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Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Intravenous Pyridostigmine and Oral Doses of Standard and Sustained-Release Pyridostigmine in Healthy Men and the Influence of Food on Oral Pyridostigmine Pharmacokinetics

TASK ORDER #14

DRAFT FINAL REPORT

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Preface

This report was prepared at The Johns Hopkins University School of Medicine, 600 North Wolfe Street, Baltimore, Maryland 21205, supported by the U.S. Army Medical Research and Development Command, Contract No. DAMD17-85-C-5133, Task Order #14, "Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Intravenous Pyridostigmine and Oral Doses of Standard and Sustained-Release Pyridostigmine in Healthy Men and the Influence of Food on Oral Pyridostigmine Pharmacokinetics." This project was conducted in collaboration with the Division of Experimental Therapeutics, Walter Reed Army Institute of Research. Col. Brian Schuster, M.D. of the Division of Experimental Therapeutics was the project monitor.

This work was conducted in The Johns Hopkins Hospital between 11 March and 26 May 1990.

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adjunct to atropine

SUMMARY

Pyridostigmine bromide may be a useful agent to prevent death from organophosphate exposure if given in advance of the exposure and if given in a dose that is adequate to inhibit red blood cell acetylcholinesterase by 20-40%. Previous studies conducted at our institution determined that single doses of pyridostigmine bromide administered as syrup inhibited red blood cell acetylcholinesterase by 20-40% longer than equivalent doses of various "sustained-release" tablets and capsules, but syrup is inconvenient in a field situation. The best experimental sustained-released formulation (45 mg capsule) kept red blood cell acetylcholinesterase inhibition above 20% for four hours. In an effort to find a formulation that would provide adequate acetylcholinesterase inhibition for a longer period, other formulations are being investigated.

This study was designed (1) to characterize the pyridostigmine bromide pharmacokinetics in healthy men after single doses of two oral dosage forms of pyridostigmine bromide, one a sustained-release preparation and the other a standard tablet, and compare these to the pharmacokinetics of a prolonged intravenous pyridostigmine infusion, (2) to characterize the time course of red blood cell acetylcholinesterase inhibition following the administration of these two oral dosage forms and compare these to the inhibition occurring with intravenous pyridostigmine, (3) to assess the effect of food on the pharmacokinetics and pharmacodynamics of the two oral pyridostigmine dosage forms, and (4) to assess the safety, tolerance, pharmacokinetics, and pharmacodynamics of multiple doses of the oral formulations over two days.

The clinical portion of the study was conducted between 11 March and 26 May 1990, and showed that the mean bioavailability of the sustained-release tablet given fasting was 8% by pharmacokinetic assessment and 8% by assessment of the inhibition of erythrocyte acetylcholinesterase when compared to intravenous pyridostigmine. The bioavailability of the standard tablet given fasting was 17% by pharmacokinetic assessment and 19% by assessment of acetylcholinesterase inhibition. The effect of food was minimal for the sustained-release formulation, shifting the acetylcholinesterase inhibition-time curve about two hours to the right. On the other hand, food caused a reduction in the degree of acetylcholinesterase inhibition following the first standard tablet with food, but this effect was absent when the inhibition-time curve at steady state with feeding was compared to the fasting dose. Both formulations were well tolerated when given in multiple doses over two days.

FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

For the protection of human subjects, the investigators have adhered to the policies of applicable Federal Law 45 CFR 46.

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1. INTRODUCTION

Studies in animals have indicated that carbamate acetylcholinesterase inhibitors have protective effects against organophosphate poisoning. Pretreatment of animals with carbamates and atropine prior to nerve agent exposure has improved survival and effectively increased the LD_{50} of nerve gas agents (1). Experimental carbamate pretreatment is only effective when used in conjunction with atropine and is not adversely affected by oximes (1-4). Of the carbamates studied, pyridostigmine bromide has been found to be a useful agent with a duration of protective action of about four hours in guinea pigs following the intramuscular route of administration (2) and as long as 24 hours following oral administration of 1/9 the LD_{50} of pyridostigmine in rabbits which were also supported by antidotal therapy (5).

The mechanism of action of pyridostigmine is thought to be carbamylation of a fraction of the tissue acetylcholinesterase, thereby protecting the enzyme from irreversible inhibition by the organophosphate (1-3, 5, 6). The relatively rapid elimination of pyridostigmine from the body (7) has the disadvantage that a single dose of pyridostigmine bromide provides only a short period of protection. Therefore, if longer periods of protection from possible organophosphate exposure are desired, either repeated administration of pyridostigmine bromide or formulations with increased and/or prolonged absorption will be required. In addition to fast elimination of pyridostigmine from the body, there is also rapid decarbamylation of the pyridostigmine-inhibited acetylcholinesterase (8), leading to a swift recovery of functional enzyme. In pyridostigmine-pretreated casualties following

exposure to a nerve agent, the rapid hydrolysis of carbamylated acetylcholinesterase restores enzyme activity in a short period of time, possibly minimizing the time period required for intensive therapy.

The dose of pyridostigmine bromide and degree of acetylcholinesterase inhibition which provide adequate protection against organophosphate poisoning while at the same time minimizing unacceptable toxicity in man are not known. Studies in rats have indicated that inhibition of twenty-five percent of blood acetylcholinesterase does not affect muscle twitch tension, even after several days of therapy. On the other hand, abnormalities in twitch tension occurred in animals treated with enough pyridostigmine to produce sixty-eight percent inhibition of the enzyme (9). In addition, ultrastructural changes at the neuromuscular junction have been seen in rat diaphragm neuromuscular junctions at doses of pyridostigmine low enough to cause only about 10% reduction in blood acetylcholinesterase levels, and more severe damage was seen with doses of pyridostigmine causing 70% reduction in acetylcholinesterase activity. Changes from single doses of large amounts of pyridostigmine were present within 24 hours and appeared to be more extensive at 7 days after dosing. In animals given lower doses for 14 days, the changes appeared to be less severe and were largely reversible based on observations on animals sacrificed 7, 14, or 23 days after drug administration ended (10).

The optimal use of pyridostigmine in man as prophylaxis for organophosphate poisoning thus presupposes that pyridostigmine is present in the body at the time of exposure and that a level of drug sufficient to provide protection while having few toxic effects can be achieved. With the fixed positive charge on the pyridostigmine molecule likely to make

transdermal delivery difficult, multiple oral doses of drug appear to be, at least for the short term, the most feasible regimen of administration for prolonged protection.

Clinical trials in healthy subjects suggest that oral pyridostigmine is generally well tolerated. In a study involving normal subjects, doses of 60 mg orally and 2, 4, and 8 mg intravenously were employed, and the most frequent symptoms reported were fatigue, fasciculations, heavy eyelids, and gastric distress (11). These symptoms usually occurred during the first 2-3 hours after dosing and were not felt to be related to plasma pyridostigmine levels. In another study conducted elsewhere and sponsored by the Army, 12 healthy subjects received 18 doses of 45 mg of syrup or more (range 45.5-72.6 mg, mean 53.4 mg). No significant clinical or laboratory adverse effects were noted, despite peak acetylcholinesterase inhibition of 32.4-66.5% (mean 52.4%) (12).

Based on the animal data, and given the lack of clinical toxicity from large pyridostigmine doses given to volunteers (11, 12), we have evaluated pyridostigmine syrup and several "sustained-release" formulations of pyridostigmine bromide in healthy subjects (13-16). Syrup is effective but its effect lasts only a few hours and it is inconvenient. The sustained-release formulations we have assessed have not been ideal in their capacity to inhibit erythrocyte acetylcholinesterase by 20% or more for periods longer than a few hours. The ideal preparation should provide more prolonged and more stable delivery of pyridostigmine and thereby result in longer periods of inhibition within the target range of 20 to 40% erythrocyte acetylcholinesterase inhibition. This study was designed to determine the

refs 13-16 ≠ ref. page

amount of pyridostigmine absorbed and the degree of erythrocyte acetylcholinesterase inhibition induced by two oral preparations of pyridostigmine bromide, one a standard tablet and the other a sustained-release preparation, compared to intravenous pyridostigmine as a reference. Intravenous pyridostigmine was chosen as the reference because it is commercially available and because our previous work has characterized its pharmacokinetics and pharmacodynamics (17). That study demonstrated the predictability and consistency of red blood cell acetylcholinesterase inhibition by a prolonged slow infusion of intravenous pyridostigmine.

2. MATERIALS AND METHODS

2.1 Pyridostigmine

Three pyridostigmine bromide preparations were used in this study. The first was intravenous pyridostigmine, produced by Hoffmann-LaRoche, Inc., diluted in normal saline. Aliquots of the infusate were assayed for pyridostigmine base concentration. Two oral pyridostigmine bromide tablets were investigated: (a) a 90 mg "sustained-release" preparation produced by the University of Iowa, and (b) a "standard" 30 mg tablet produced by Duphar, a subsidiary of Kali-Duphar Laboratories, Inc. The tablets arrived at our facility on 08 March 1990. All test medications were provided by the U.S. Army. The supplies were kept in secure areas under lock and key in the Clinical Pharmacology Division complex of The Johns Hopkins Hospital or in the Pharmacy Department of The Johns Hopkins Hospital.

The intravenous pyridostigmine was diluted with normal saline to a total volume of 24 ml and infused at a constant rate by an infusion pump. The tablets were administered intact to the subjects and the subjects were noted

to swallow the tablets with water. The amount of pyridostigmine bromide administered to each subject is given in Tables 1, which is based on the results of the assays of each of the formulations conducted at SRI International under Contract No. DAMD17-85-C-5141 (13).

2.2 Subjects

Healthy men who were able to give written informed consent were eligible to volunteer for the study. The study was approved by the Joint Committee on Clinical Investigation of The Johns Hopkins Medical Institutions and the Human Subjects Research Review Board of the U.S. Army.

2.2.1 Inclusion Criteria

To participate in the study the volunteer had to be male, between 18 and 35 years of age, and was to be within 10% of his ideal body weight as determined by Metropolitan Life Insurance Company tables (14). Approximately equal numbers of whites and non-whites were to be recruited. Each subject was demonstrated to be in good general health based on a detailed health history and physical examination performed by a physician. Serum chemistries, hematology tests, and urine analysis had to be within normal ranges, as defined by The Johns Hopkins Hospital Department of Laboratory Medicine. The protocol provided that the creatine kinase (CK) could be above the "normal range" and not exclude the subject because of the frequent finding of elevated CK in healthy subjects who are especially active physically (15-20). Nevertheless, at the request of the Army monitor for this study, the normal range according to the Hopkins laboratory was used as the acceptable range for the subjects in this study. An electrocardiogram within 12 months of entry had to be normal. The remainder of the screening evaluation was

completed within 14 days of entry into the study.

2.2.2 Exclusion Criteria

Women were excluded from this study. Men were excluded if they did not meet the criteria listed above (2.2.1) or if they had a known or suspected allergy to pyridostigmine bromide or related drugs. Those with a history of significant heart disease, asthma, or other respiratory disorders were excluded. Once accepted as candidates for the study, subjects were not permitted to take any medication for one week prior to admission to the study. A positive drug screen for opioids or cocaine on the day of admission excluded the volunteer.

2.2.3 Recruitment

Advertisements were placed in the help wanted classified sections of metropolitan Baltimore newspapers. A special telephone line was dedicated to volunteer recruitment. Interested candidates were screened on the telephone by a recruiter/screener who described the details of the study, took a brief history and scheduled the appropriate screening examinations.

2.2.4 Informed Consent

Written informed consent was obtained from each participant upon admission to the Clinical Research Unit. The consent document described in detail the purpose of the study, the research protocol, and the potential risks (Appendix A).

2.2.5 Compensation

A payment schedule was designed to compensate volunteers for their participation based on the number of days they were confined to the

research unit, the number of doses of test medication given, and the number of blood samples taken. Each of the volunteers participating in Task Order #14 was remunerated $350. Eight payments of $10 each were made to subjects who returned at our request for follow-up laboratory testing after discharge values returned abnormal. One individual received a $10 finder's fee. For the entire study, $5,690.00 was distributed to the volunteers.

2.2.6 Liability

Liability coverage for unexpected toxicity was provided by the U.S. Army, and for malpractice by The Johns Hopkins Medical Institutions.

2.3 Experimental Protocol

2.3.1 Objectives

There were four objectives of the study: (1) to characterize the pharmacokinetic profiles of two oral pyridostigmine dosage forms, one a sustained-release preparation and the other a standard tablet, and compare these to the pharmacokinetics of a prolonged intravenous pyridostigmine infusion; (2) to characterize the time course of RBC AChE inhibition following the administration of these two oral dosage forms and compare these to the inhibition occurring with intravenous pyridostigmine; (3) to assess the effect of food on the pharmacokinetics and pharmacodynamics of the two oral pyridostigmine dosage forms; and (4) to assess the safety, tolerance, pharmacokinetics and pharmacodynamics of multiple doses of the oral formulation over two days.

2.3.2 Design

The study was conducted as an open design study. The morning after admission (Day 2), each subject received intravenous pyridostigmine bromide, 6000 micrograms in 24 ml normal saline (250 mcg/ml), over 6 hours by Harvard pump. The next morning (Day 3) a single dose of one of the two pyridostigmine bromide tablets was given, and then two days later (Day 5) the subjects began two days of multiple dosing with the same tablet. The first eight subjects were to receive the sustained-release tablet and the next eight were to receive the standard tablet. The intravenous dose was always given first, the single oral dose given the next day, and the multiple-dose portion started two days after the single dose. For the intravenous and single-dose oral administration (Days 2 and 3), the subjects were fasted except for water and also not allowed to smoke for 8 hours before the dose and for 4 hours after the dose, when they were allowed to eat and to smoke if they desired. On Day 5, concurrently with the first dose in the multiple-dose portion, the subjects ate a standard breakfast consisting of 8 fl. oz. of 2% milk, 4 fl. oz. of orange juice, two slices of wheat toast with margarine and jelly, and two scrambled eggs. Following drug administration serial blood specimens were obtained at times specified in the protocol, and non-directed questioning regarding the development of any symptoms was used to monitor for clinical adverse reactions. The outline of the study and the details of how it was to be conducted as initially planned are contained in the Study Flow Sheet (Appendix B). All subjects were screened outside the hospital. Drug administration, sample collection, and post-drug toxicology monitoring were performed in the Drug Development Unit/Clinical Research Center of The Johns

Hopkins Hospital.

2.4 Clinical Laboratory Examination

All laboratory examinations except for assay of pyridostigmine were done within The Johns Hopkins Medical Institutions. Hematology and chemistry determinations were performed by the Department of Laboratory Medicine (Clinical Laboratory License number 19-1054). The normal values for these determinations are listed in Appendix C. Erythrocyte acetylcholinesterase assays were performed in the research laboratory of the Division of Clinical Pharmacology (see section 2.6.2). The clinical hematology and chemistry tests were performed at screening, upon admission to the hospital, and on the morning of discharge to monitor for safety. In addition, creatine kinase was measured on a specimen collected at 8 hours on each day of drug administration. If the results of the laboratory tests performed on the morning of discharge were abnormal, the subjects were to return at weekly intervals to repeat the abnormal tests until the values returned to normal or an alternative explanation for the abnormalities was determined.

2.4.1 Hematology

Routine hematologic determinations, including hematocrit, hemoglobin, red blood cell count, white blood cell count with differential count, and platelet count were done with a Coulter^{R} counter.

2.4.2 Chemistry

Serum was assayed for sodium, potassium, chloride, carbon dioxide, urea nitrogen, creatinine, glucose, uric acid, calcium, phosphate,

total protein, albumin, cholesterol, direct and total bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and creatine kinase.

2.4.3 Electrocardiography

Standard 12-lead electrocardiographic tracings were usually taken just prior to admission to the hospital, and in all cases within 12 months of entry. Electrocardiograms were interpreted by a physician on the staff of The Johns Hopkins Hospital who has formally read electrocardiograms for hospitals for 10 years.

2.4.4 Urine Analysis

Urine analysis was performed in the laboratories of the Division of Clinical Pharmacology. Protein, ketones and bilirubin were measured qualitatively, and pH and specific gravity were quantitatively determined. A microscopic examination of the sediment was also performed.

2.5 Specimen Handling

2.5.1 Blood Collection and Storage

Most blood specimens were obtained by means of a heparin lock inserted prior to drug administration, though occasionally blood was obtained by venipuncture. During the intravenous pyridostigmine infusion, specimens were obtained from the arm contralateral to the infusion. Specimens for pyridostigmine concentration and erythrocyte acetylcholinesterase determination were handled as described in section 2.6. Routine chemistry and hematology clinical specimens were placed in appropriate Vacutainer^{R} tubes and sent to the hospital's Clinical Laboratory for analysis.

2.5.2 Specimen Shipment

The frozen plasma and blood specimens were shipped in a sealed insulated container packed with dry ice. Shipment was made by an overnight carrier to Dr. Emil Lin at the University of California at San Francisco.

2.6 Pyridostigmine and Erythrocyte Acetylcholinesterase Determinations

2.6.1 Pyridostigmine Analysis

Blood samples of 5 ml each for pyridostigmine assay were obtained in plastic syringes, placed in heparinized Vacutainer^{R} tubes, and inverted to insure adequate mixing. The samples were then promptly placed in ice water for transport to our laboratory. As soon as possible (generally within five minutes), the samples were centrifuged for ten minutes in a refrigerated centrifuge. The plasma was separated, transferred to labelled plastic containers, frozen in acetone-dry ice, and stored at -80°C until it was shipped in dry ice to the assay site. The entire process from blood drawing to freezing was less than 20 minutes.

The assay of pyridostigmine in plasma was performed under contract DAMD17-6-C-6150, USAMRDC, in the laboratory of Dr. Emil Lin at the School of Pharmacy, University of California at San Francisco (21). The assay utilizes protein precipitation with acetonitrile, pre-column purification on a small C8 Bond Elut^{R} column, and then high performance liquid chromatography on a silica column with ultraviolet detection. The assay is sensitive to 1.6 ng/ml of pyridostigmine bromide. Accuracy is 8 to 12% in the concentration range of 0 to 50 ng/ml. Precision is 3 to 14% (21). The plasma

pyridostigmine concentrations of the subjects in this study are listed in that report.

2.6.2 Erythrocyte Acetylcholinesterase Determinations

Blood samples for erythrocyte acetylcholinesterase determinations (12 ml at baseline and 3 ml each time thereafter) were obtained at the same times as the plasma pyridostigmine samples. The blood was obtained in plastic syringes, transferred into Vacutainer^{R} tubes containing ethylenediaminetetraacetic acid (EDTA), mixed immediately and brought to the Clinical Pharmacology laboratories for immediate assay -- generally within five minutes of collection. These specimens were kept at ambient temperature until assayed. The assay was performed according to the Standard Operating Procedure (SOP) for the assay of the Analytical Chemistry Branch, USAMRICD, Aberdeen Proving Ground, Maryland 21010 dated 18 June 1985 (22). As performed at Johns Hopkins, the assay is linear between 2.94 and 14.70 uM/ml/min of product produced, with a coefficient of variation determined from the quality control standard less than 2%. Details of the assay and its performance at Johns Hopkins are contained in Appendix D.

2.7 Pharmacokinetic and Pharmacodynamic Analyses

2.7.1 Pharmacokinetic Analysis

Visual inspection of the pyridostigmine plasma concentration time-curves following the administration of intravenous pyridostigmine revealed a rise in the pyridostigmine concentration over the first three hours, followed by a plateau for the next three hours of intravenous administration, and then a decline after the infusion was discontinued. A manual graphic analysis of the decline in concentrations

following the end of the infusion was performed. A biphasic decline occurred in most subjects. Macro- and micro-elimination rate constants were estimated and the values used as initial estimates for the curve fitting process. PCNONLIN^{R}, a commercially available program for the estimation of pharmacokinetic parameters, was used to estimate the variables which best fit the observed data to a two-compartment model (23). Data points were weighted to the reciprocal of the value because this appeared to be the best method of estimating both the high and the low plasma pyridostigmine concentrations. This parameter estimation process produced estimates for the rate constant of elimination, K_{10}; the intercompartmental rate constants, K_{12} and K_{21}; and the volume of the central compartment, V. The Nelder-Mead method was used to obtain the best parameter estimates.

Inspection of the pyridostigmine plasma concentration-time curves after the oral administrations, both fed and fasted, revealed a short lag period followed by a gradual rise in concentration and then a slow decline. This shape suggested that absorption was occurring gradually. A two-compartment model with first-order absorption after a lag time and first-order elimination was assumed. In addition, it was assumed that the estimates of the pharmacokinetic parameters for each subject obtained from the intravenous data were unchanged. The estimates for the parameters in the intravenous fit were thus employed as constants for fitting the oral data. Data from the dose given fasting and the initial dose administered with food were analyzed separately. Best parameter estimates were obtained for the following: the time lag before the onset of absorption, TLAG; the rate constant of absorption, K_{01}; and the bioavailability, F, for both the fasted

and first-fed dose. As before, a weighted least squares approach using the Nelder-Mead method was employed and data points were weighted to the reciprocal of the observed value. Examples of the instruction files for the parameter estimation are included as Appendix E.

Inspection of the trapezoidal rule areas under the concentration-time curves revealed that the ratio of the "steady state" fed area to the first-dose fed area was highly variable and sometimes less than unity, suggesting that the fraction absorbed (and possibly K_{01} and TLAG as well) was not constant over all doses. Therefore, no pharmacokinetic analysis of the data obtained during the multiple-dose administration while the subjects were eating was performed.

2.7.2 Pharmacodynamic Modeling

An evaluation of pyridostigmine effect (erythrocyte acetylcholinesterase inhibition or activity) was conducted for each intravenous dose, each dose of oral pyridostigmine given fasting, and the first dose each morning in the multiple-dose portion of the study. The degree and duration of acetylcholinesterase inhibition was determined, and the areas under the inhibition-time curves were compared.

2.8 Statistical Methods

Estimates of the subject population parameters and the mean and standard deviation of the calculated variables have been determined using standard formulae (28).

3. RESULTS

3.1 Amendments and Compliance

3.1.1 Amendments

Rather than giving the sustained-release tablet to the first eight subjects and the standard tablet to the next eight subjects, the participants were dosed in pairs, alternating between the standard tablet and the sustained-release tablet, with the first two receiving the standard tablet, the next two the sustained-release tablet, and so on. This change was made to avoid a "period effect," which could have developed by studying all of the subjects in one arm over a relatively short period of time and then studying all of the subjects in the other arm over a subsequent period of time. A period effect raises the possibility that technical aspects of the study could vary from the first period to the second, mistakenly suggesting a difference between the two tablets. Intermingling the pairs of subjects in the two study arms would minimize or eliminate any such period effect. This change was made in collaboration with the study monitor.

3.1.2 Compliance

Only one subject who was enrolled into the study did not fall within 10% of the ideal weights for height as described in the Metropolitan Life Insurance Company tables. This subject was one pound "underweight." This subject was allowed to enter the study because his weight was stable and he was by all other measures completely healthy. Two other subjects were of a height greater than that provided in the tables, so extrapolation of the tables was required and the subjects were allowed to enter the study. These were Subjects #13 (203.5 cm, 83.4 kg) and Subject #15

(197.0 cm, 100.3 kg).

The final protocol was not consistent regarding chest x-rays within 12 months of entry into the study as part of screening. They were called for in one portion of the narrative, but not in another portion and not on the Study Flow Sheet. In addition, chest x-rays were required in previous task orders where pyridostigmine was studied, and they were required for another Army study which we were conducting concurrently. In this study, standard posteroanterior and lateral chest x-rays were performed on all subjects within 12 months of entry into the study. The x-rays were interpreted by members of the Department of Radiology of the Johns Hopkins Hospital. Nine of the sixteen subjects had chest x-rays performed just before entry into this study which appeared to have been done specifically for this study. The other seven subjects had normal chest x-rays within the preceding 12 months, so repeat x-rays for this study were not performed.

There were occasional deviations of actual sample collection from the scheduled sampling times during the course of the project. These deviations were usually a matter of only minutes, though some of the 24-hour samples were obtained as early as 2.5 hours before the scheduled time. The minor deviations were usually due to difficulties with blood drawing. The earliest specimen collections at 24 hours were due to delays in standardizing the equipment before the dose was given, moving up the "24-hour" sample which was collected the next day approximately on time according to the original schedule. The longest delays at 24 hours (approximately 30 minutes) were due to problems with standardizing the instrument used to assay acetylcholinesterase first thing in the morning; blood samples were not obtained until the

machine was standardized and working properly. All of the deviations of sample collection from the prescribed times can be found in Tables 2-9. All calculations from the results obtained in this study are based on actual times of sample collection, not scheduled times.

3.2 Description of Population of Subjects

Sixty-nine different men, 18-35 years of age, were screened for participation in the study once or more using criteria outlined in Section III. A. and B. of the protocol. Individuals were screened more than once if their initial abnormalities seemed minor and might have resolved after a period of days to weeks, enabling them to enter the study if the abnormalities had resolved upon repeat testing. From this pool, sixteen subjects who met the laboratory criteria and passed the history and physical examination were chosen to participate in the inpatient study. Reasons for rejection among the 53 individuals assessed who failed the screening evaluation included the following:

(a) 30 volunteers were rejected for elevated creatine kinase levels;

(b) 4 volunteers were rejected for high white blood cell counts;

(c) 1 volunteer was rejected for low white blood cell count;

(d) 7 volunteers were rejected for low hematocrits;

(e) 38 volunteers were rejected for elevated serum levels of hepatic transferases; and

(f) 2 volunteers failed the history and/or physical examination.

This summary totals more than 53 volunteers because some failed more than one test and some were screened more than once and failed for the same or different reasons.

The sixteen volunteers meeting laboratory testing criteria and passing the history and physical examination were admitted to The Johns Hopkins Hospital and entered into the study. Of the sixteen who successfully completed the study, nine were white and seven were black. The average age was 27 years and ranged from 20 to 35 years. Relevant vital statistics of these volunteers are listed in Table 10.

3.3 Clinical Results

3.3.1 Symptomatic

During the intravenous infusion day, three subjects (Subjects #5, #9, and #15) developed gastrointestinal complaints, specifically, nausea and/or flatulence with or without abdominal pain. Of interest, these were not the smallest subjects (in other words, not the subjects with the highest dose per kg body weight). In fact, Subject #15 was the heaviest of all the subjects. These symptoms also developed in Subjects #5 and #15 with the single oral dose given fasting. All these symptoms resolved spontaneously. Subject #7 complained of nausea and diarrhea on the second day of multiple dosing. These symptoms were improved but not totally resolved the next morning at the time of discharge. Another subject (Subject #8) complained of "rumbling in the stomach" or "hunger pangs" on the first evening of multiple dosing. This occurred while he was showering, improved

with sitting down, and lasted only about 15 minutes, without recurrence. Two subjects (Subjects #7 and #9) developed headaches during the multiple-dose portion of the study, in one case severe enough to require acetaminophen for relief. One subject (Subject #2) complained of ringing in the ears lasting about 10 minutes, starting about an hour after the first dose of oral medication given with food. He did not complain about it again. Another subject (Subject #3) developed a slightly raised, erythematous rash associated with dryness and scaling of the skin, localized to the forehead. This eruption began on the second afternoon of multiple dosing. This sort of rash had occurred before in this individual, he attributed it to soaps or lotions used, and it resolved overnight. One subject (Subject #6) broke a tooth while eating his lunch on the day of intravenous drug administration. Even though this event occurred after the test drug had been started, we could not conceive of any biologically plausible explanation for how the drug may have contributed to this event, and therefore it was not attributed to the drug. A summary of these symptomatic complaints was provided to Army personnel on 02 August 1990 in response to their request, and the summary is provided as Appendix F.

3.3.2 Vital Signs

None of the subjects had a clinically significant change in temperature, blood pressure, heart rate, or respiratory rate following the administration of any of the pyridostigmine bromide doses.

3.3.3 Laboratory

3.3.3.1 Liver Function Tests

Three subjects developed slight abnormalities of

liver function tests during the course of the study. These subjects were Subjects #2, #14, and #15. In each case the alanine aminotransferase level was above the upper limit of normal (30 units/liter) only on the day of discharge. The elevations ranged from 35-72 units/liter. On the same dates the aspartate aminotransferase levels were still normal in two of these subjects, but slightly elevated (42 units/liter) in Subject #14, who had the highest alanine aminotransferase level (72 units/liter). None of these subjects had any symptoms of hepatic dysfunction and were discharged as scheduled per protocol. Efforts were made to have the subjects return the next week for follow-up studies, but only Subject #2 returned, and his follow-up values were normal.

3.3.3.2 Creatine Kinase

The creatine kinase level was normal in all subjects at screening with the exception of Subject #15, whose level of 278 mg/dl was above the upper limit of normal (160 mg/dl) at the time of screening. This subject's creatine kinase level was normal at 123 mg/dl four days later at the time of admission, and remained normal throughout the study. One other volunteer (Subject #8) had a normal creatine kinase level at screening but an elevated level (295 mg/dl) at the time of admission. His creatine kinase level the next morning, prior to administration of any test drug, was normal at 133 mg/dl, and it remained normal throughout the remainder of the study. The creatine kinase did not increase above the normal range in any of the subjects.

3.3.3.3 Other Clinical Chemistry Tests

No other clinical chemistry tests showed any

significant changes over the duration of the protocol.

3.3.3.4 Hematological Testing

All subjects had normal hematological values at the time of admission. Subject #10 was slightly anemic at screening (40.5%) but normal at the time of admission (42.3%). All sixteen subjects had a fall in hematocrit during the course of the study. In 11 of the 16 subjects the fall was greater than 5% from the baseline values. These declines were probably due to blood loss via venipuncture, but a drug effect could not be completely excluded.

3.3.3.5 Electrocardiograms

All subjects were found to have electrocardiograms free of any evidence of clinically significant abnormalities. Repeat electrocardiograms were not obtained, which was in accordance with the specifications of the protocol.

3.3.4 Clinical Conclusions

The subjects tolerated the administration of the test drug and their hospitalization with frequent blood drawing fairly well. Gastrointestinal complaints were most frequent, including flatulence, nausea, abdominal pain, and diarrhea, but were usually short-lived and spontaneously reversible. The adverse events observed in this study are summarized in Table 11, and the criteria used to categorize their relationship to the drug are provided in Appendix G.

3.4 Pharmacokinetics and Pharmacodynamics

3.4.1 Pharmacokinetics

The concentration of pyridostigmine expressed in nanograms of base per ml of plasma is given in Tables 12-19. The intravenous administrations produced rising concentrations up to three hours, followed by a plateau for the next three hours, and then falling concentrations after the administration was stopped after six hours. Mean concentrations in the two groups were nearly identical, suggesting that the pharmacokinetic parameters for the two groups of subjects are similar.

Plasma concentration data were used to fit a two-compartment model. The resulting best estimates for the intravenous pharmacokinetic parameters are displayed in Tables 20 and 21. The model seems to be a good one for all subjects except subjects #13, #14, and #16 in whom long beta half-lives with larger uncertainty were estimated suggesting that a one-compartment model may have fit the data better. Mean pharmacokinetic constants for the two groups are similar as suggested by the concentrations.

It is apparent that the sustained-release pyridostigmine tablet was associated with earlier detection of pyridostigmine base in the plasma, higher peak concentrations and longer periods of detectable plasma levels than the standard pyridostigmine tablet. However, the dose of sustained-release pyridostigmine was 90 mg compared to 30 mg in the standard tablet. The T_{max} for the sustained-release pyridostigmine was greater than T_{max} for the standard pyridostigmine tablet, suggesting that absorption was occurring at later times after dosing with the sustained-release preparation. Taking the preparations with food delayed the T_{max} for both preparations.

As suggested by the concentration data, the mean half-life of absorption for the sustained-release tablet is larger than that of the

standard tablet in both the fasted and fed states. For both preparations, feeding prolonged absorption; the time lag before absorption was increased and the half-life lengthened when pyridostigmine was administered with food. Estimates of the pharmacokinetic parameters for each subject are displayed in Tables 22 and 23. For both oral preparations we observed two- to fourfold intersubject variations for most parameters except the lag times for absorption.

The areas under the pyridostigmine concentration-time curve (AUC) were calculated for the intravenous infusion and for both of the oral formulations using the model-independent method of the trapezoidal rule to the last measured concentration. The calculated areas under the concentration-time curve and resulting bioavailability of the two oral preparations are displayed in Table 24. As suggested by the nearly superimposable plasma concentrations in the two groups, the mean areas under the pyridostigmine concentration-time curve after the intravenous pyridostigmine were nearly identical, implying quantitatively similar disposition of systemic drug in the two groups. Differences in the AUC's following oral drug are thus due to differences in the size of the dose and/or the absorption characteristics of the preparations. Areas under the concentration-time curve were generally higher for the sustained-release tablet than for the standard tablet. When adjusted for the different dosing schedules, at steady state the sustained-release tablet produced a mean daily AUC 40% higher than that of the standard tablet (2 x 217 ng·hr/ml vs 3 x 103 ng·hr/ml, respectively).

During the multiple-dose portion of the study, the AUC was higher at steady state compared to the first dose in all subjects who

received the standard tablet. In contrast, three of eight subjects who received the sustained-release tablet had lower AUC's at steady state than during the first dose, suggesting erratic absorption of this preparation.

Mean bioavailability was higher for the standard tablet than the sustained-release tablet, 17% vs. 8% when fasted and 18% vs. 12% when administered with food. Food did not affect the bioavailability of either the standard tablet (17% fasted, 18% fed; $p>0.05$) or the sustained-release tablet (8% fasted, 12% fed; $p = 0.06$, Wilcoxon rank sum test).

3.4.2 Pharmacodynamics

The degrees of inhibition of erythrocyte acetylcholinesterase activity after the various doses of intravenous and oral pyridostigmine bromide are shown in Tables 25-32. The inhibition induced by the continuous intravenous infusion of pyridostigmine is shown in Tables 25-26, and reveals that inhibition increased over the first 3-5 hours and then plateaus until the infusion was stopped at 6 hours, whereupon the inhibition steadily fell over the next 6-8 hours. The time course of inhibition was similar in both groups of subjects (Figure 1). The measurable effect of the sustained-release tablet was slightly earlier than with the standard tablet given fasting and the degree of inhibition was greater with the sustained-release tablet compared to the standard tablet (Figure 2). However, the pyridostigmine dose in the sustained-release tablet was three times greater than the standard tablet and the two oral formulations were tested in different subjects. (When adjusted for dose of pyridostigmine administered, the standard tablet was more efficacious than the sustained-release tablet in acetylcholinesterase inhibition.) The administration of the standard tablet

with food appeared to decrease the extent of acetylcholinesterase inhibition compared to fasting (Figure 3). On the other hand, the administration of the sustained-release tablet with food appeared to have little effect on the mean peak inhibition, but shifted the inhibition curve to the right, causing prolongation of the inhibition observed (Figure 4). The multiple-dose assessment of inhibition demonstrated that the mean peak inhibition for both tablets was at 3.0-3.5 hours after dosing. Mean inhibition was greater at steady state throughout the dosage interval with the sustained-release tablet as compared to the standard tablet (Figure 5). Mean inhibition 12 hours after dosing with the sustained-release tablet was the same as mean inhibition 8 hours after the standard tablet.

The bioavailability of the two oral formulations determined from the inhibition data is shown in Table 33. These estimates are very close to those determined using pharmacokinetic data (Table 24). The mean bioavailability of the standard tablet given fasting was 19%, and 19% at steady state in the multiple-dose portion. The mean bioavailability of the sustained-release tablet was 8% while given fasting and 11% at steady state in the multiple-dose portion.

Tables 34-41 show the periods when erythrocyte acetylcholinesterase inhibition exceeded 20% and 40% after dosing with the intravenous and oral formulations. The intravenous infusion was excellent in its capacity to achieve 20-40% target range, exceeding 40% in only one of 16 subjects. The duration that the inhibition exceeded 20% for the intravenous infusion was 4.0-6.0 hours. Despite the difference in pyridostigmine dose, the mean duration of inhibition greater than 20% was not substantially

different between the standard and sustained-release tablets given fasting (4.3 vs. 4.64 hours, respectively). Three of eight subjects receiving the standard tablet and 6 of 8 receiving the sustained-release tablet exceeded 40% inhibition when the formulations were given fasting. When the standard tablet was given with food, the duration of inhibition greater than 20% was less in 6 of 8 subjects than when the dose was given fasting, and the mean duration was 3.40 hours with food compared to 4.31 hours when the dose was given fasting. On the other hand, when the sustained-release tablet was given with food, the duration of inhibition greater than 20% was increased in 6 of 8 subjects and the mean duration of each inhibition increased from 4.64 hours when the dose was given fasting to 6.27 hours when the dose was given with food. When given with food, the inhibition never exceeded 40% with the standard tablet, but exceeded 40% in 5 of 8 subjects with the sustained-release formulation when given with food.

The mean duration of inhibition greter than 20% during the multiple-dose portion was 5.58 hours with standard tablet compared to 9.14 with the sustained-release tablet. This represents 70% of the 8-hour dosing interval for the standard tablet and 76% of the 12-hour dosing interval for the sustained-release tablet. The inhibition during the multiple-dose assessment reached 40% at only one time point in two of the eight subjects receiving the standard tablets. On the other hand, the indibition during the multiple-dose assessment of the sustained-release table exceeded 40% in five of the eight subjects, and the mean duration of inhibition greater than 40% during the multiple-dose assessment of the sustained-release tablet was 2.07 hours (17% of the dosage interval).

4. DISCUSSION

The results of this study indicate that both of the oral formulations had low bioavailability, whether determined by pharmacokinetic or pharmacodynamic assessment compared to intravenous pyridostigmine. The bioavailability determined by these two methods was in good agreement. Of the two oral formulations, the standard tablet had a bioavailability twice that of the sustained-released formulation when both were given fasting, and 50% greater bioavailability when both were given in the multiple-dose portion of the study.

Because of the higher dose of pyridostigmine in the sustained-release tablet, the desired effect of the drug the duration of (erythrocyte acetylcholinesterase inhibition greater than 20%) was more prolonged than with the standard tablet. Nevertheless, the duration of inhibition greater than 20% did not extend throughout the dosing interval during the multiple-dose portion with either formulation. With the standard tablet the duration of inhibition greater than 20% was 70% of the dosing interval, while with the sustained-release tablet it was 76%. These are not statistically different. On the other hand, erythrocyte acetylcholinesterase inhibition was greater than 40% during 17% of the dosage interval during multiple dosing with the sustained-release tablet compared to only one time point in two subjects while dosing with the standard tablet. To the extent that excess inhibition causes symptoms or signs, the sustained-release tablet may be inferior to the standard tablet. This must be balanced against the increased convenience of taking a tablet every 12 hours as opposed to every 8 hours. Adverse events could be studied in a larger scale clinical study

comparing the two formulations and dosing regimens. Therefore it seems that either product would be a reasonable choice to use in the field. There were no significant differences in tolerance of the two preparations.

Our data suggest that the mean systemic availability of both tablets in man is less than that observed for pyridostigmine syrup or a different extended-release tablet in beagle dogs (30). In the dogs the syrup was 44% bioavailable, and the relative bioavailability of the extended-release tablets compared to syrup in the dogs was 75%, or 33% absolute bioavailability. While the dog clearly is not a useful model for predicting the extent of absorption of pyridostigmine in humans, it is still useful for studying relative bioavailabilities of various preparations. The sustained-release tablet had diminished bioavailability compared to the tablet in both species.

5. CONCLUSIONS

The results of this study indicate that the two tablet formulations were nearly equivalent in their capacity to inhibit erythrocyte acetylcholinesterase to greater than 20% during the multiple-dose portion of the study. We believe that either tablet may have clinical utility. Both formulations were well tolerated in this study with a small number of subjects dosed for a limited period. Differences in adverse event profiles of the two formulations could be evaluated in a wide-scale clinical trial assessing clinical signs and symptoms developing during the two regimens.

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Table 1

Amount of Pyridostigmine Base (mg) Administered to Each Subject

Subject	Intravenous Dose	Oral Dose
1	3.984	20.889
2	4.272	20.889
3	4.008	64.056
4	4.176	64.056
5	4.008	20.889
6	3.936	20.889
7	4.488	64.056
8	4.224	64.056
9	4.272	20.889
10	4.248	20.889
11	4.440	64.056
12	4.440	64.056
13	4.368	20.889
14	4.440	20.889
15	4.512	64.056
16	4.368	64.056

Actual Times of Sample Collection after
Intravenous Infusion in Subjects Receiving
the Standard Tablet

Scheduled Interval	Subject 01	02	05	06	09	10	13	14
	Actual Time of Sample (Hours After Dosing)							
0.00	-0.22	-0.58	-0.75	-0.83	-0.25	-0.35	-0.50	-0.33
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.50	0.50	0.50	0.50	0.50	0.50	0.65	0.50	0.50
1.00	1.00	1.00	1.00	1.00	1.00	1.03	1.00	1.00
1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25
6.50	6.50	6.50	6.50	6.50	6.50	6.50	6.50	6.50
7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50
8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
14.00			14.00	14.00	14.00	14.00	13.00	13.00

Table 2

Table 3

Actual Times of Sample Collection after Intravenous Infusion in Subjects Receiving the Sustained-Release Tablet

Scheduled Interval	Subject 03	04	07	08	11	12	15	16
	Actual Time of Sample (Hours After Dosing)							
0.00	-0.58	-0.50	-0.63	-0.58	-1.27	-1.28	-0.50	-0.42
0.25	0.25	0.25	0.25	0.42	0.25	0.25	0.25	0.25
0.50	0.50	0.58	0.50	0.50	0.50	0.50	0.50	0.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.33	6.25
6.50	6.50	6.50	6.50	6.50	6.50	6.50	6.50	6.50
7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
7.50			7.50	7.50	7.48	7.50	7.50	7.50
8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
14.00	13.83	14.08	14.00	14.00				

Table 4

Actual Times of Sample Collection in Subjects
after Receiving One Standard Tablet While Fasting

Scheduled Interval	Subject 01	02	05	06	09	10	13	14
	Actual Time of Sample (Hours After Dosing)							
0.00	-0.42	-0.42	-0.38	-0.35	-0.42	-0.42	-0.42	-0.42
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1.33	1.33	1.33	1.33	1.33	1.33	1.37	1.33	1.33
1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67
2.00	2.00	2.00	2.00	2.00	2.00	2.00	1.92	1.92
2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
3.50	3.58	3.58	3.50	3.50	3.50	3.50	3.58	3.55
4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
5.00	5.00	5.03	5.00	5.00	5.00	5.00	5.00	5.00
6.00	6.00	6.05	6.00	6.00	6.00	6.00	6.00	6.00
7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.03
10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
12.00	12.08	12.08	12.00	12.00	12.00	12.00	12.05	12.00
14.00	14.08		14.00	14.00	14.00	14.00	14.07	14.00
24.00	21.50	21.50	24.00	24.00	24.00	24.00	24.00	24.00

Table 5

Actual Times of Sample Collection in Subjects
after Receiving One Sustained-Release Tablet
While Fasting

Scheduled Interval	Subject 03	04	07	08	11	12	15	16
	Actual Time of Sample (Hours After Dosing)							
0.00	-0.42	-0.42	-0.33	-0.25	-0.50	-0.50	-0.33	-0.25
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.30
0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.55	0.50
0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
1.00	1.00	1.00	1.00	1.02	1.00	1.00	1.00	1.00
1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.35
1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67
2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
2.50	2.58	2.50	2.50	2.50	2.50	2.50	2.50	2.52
3.00	3.00	3.00	3.00	3.03	3.00	3.00	3.00	3.00
3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
5.00	5.17	5.00	5.00	5.00	5.00	5.00	5.00	5.00
6.00	6.02	6.00	6.00	6.00	6.00	6.00	6.00	6.00
7.00	7.03	7.03	7.00	7.00	7.00	7.00	7.00	7.00
8.00	8.03	8.03	8.00	8.00	8.00	8.00	8.00	8.00
10.00	10.03	10.03	10.00	10.00	10.00	10.00	10.00	10.00
12.00	12.07	12.08	12.00	12.00	12.00	12.00	12.00	12.00
14.00	14.08	14.08	14.00	14.00	14.00	14.00	14.00	14.00
24.00	24.08	24.08	24.08	24.08	24.27	24.33	24.00	24.00

Table 6

Actual Times of Sample Collection in Subjects
after Receiving One Standard Tablet with Food -- First Dose

Scheduled Interval	Subject 01	02	05	06	09	10	13	14
	Actual Time of Sample (Hours After Dosing)							
0.00	-0.25	-0.25	-0.42	-0.50	-0.42	-0.42	-0.17	-0.08
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33
1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67
2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
3.50	3.45	3.50	3.50	3.50	3.50	3.50	3.50	3.50
4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.05	7.00
8.00	8.00	7.92	8.00	8.00	8.00	8.00	8.00	8.00

Table 7

Actual Times of Sample Collection in Subjects
after Receiving One Sustained-Release Tablet
with Food -- First Dose

Scheduled Interval	Subject 03	04	07	08	11	12	15	16
	Actual Time of Sample (Hours After Dosing)							
0.00	-0.50	-0.50	-0.50	-0.50	-0.77	-0.75	-0.25	-0.25
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
0.75	0.75	0.92	0.75	0.75	0.75	0.75	0.75	0.75
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33
1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67
2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
2.50	2.50	2.50	2.50	2.50	2.58	2.50	2.50	2.50
3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
8.00	8.00	8.00	8.00	8.20	8.00	8.00	8.00	8.00
10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00

Table 8

Actual Times of Sample Collection in Subjects after Receiving One Standard Tablet on Day 2 of Multiple-Dose Portion

Scheduled Interval	Subject 01	02	05	06	09	10	13	14
	Actual Time of Sample (Hours After Dosing)							
0.00	-0.42	-0.40	0.00	0.00	0.00	0.00	-0.50	-0.45
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33
1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67
2.00	2.00	2.00	2.00	2.00	2.17	2.00	2.00	2.00
2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
3.00	3.12	3.00	3.00	3.00	3.00	3.00	3.00	3.00
3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
4.00	4.13	4.00	4.00	4.00	4.00	4.00	4.00	4.00
5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
6.00	6.13	6.00	6.00	6.00	6.00	6.00	6.00	6.00
7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
24.00	24.00	24.00	24.00	24.00	24.33	24.00	24.00	24.00

Actual Times of Sample Collection in Subjects after Receiving One Sustained-Release Tablet on Day 2 of Multiple-Dose Portion

Scheduled Interval	Subject 03	04	07	08	11	12	15	16
	Actual Time of Sample (Hours After Dosing)							
0.00	-0.50	-0.50	-0.08	0.00	-0.17	-0.17	-0.17	-0.17
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
0.75	0.75	0.75	0.83	0.78	0.75	0.75	0.75	0.75
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1.33	1.33	1.42	1.33	1.33	1.40	1.37	1.33	1.33
1.67	1.67	1.78	1.67	1.67	1.67	1.67	1.67	1.67
2.00	2.00	2.10	2.00	2.00	2.00	2.00	2.00	2.00
2.50	2.50	2.53	2.50	2.50	2.50	2.50	2.50	2.50
3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
7.00	7.00	7.00	7.00	7.08	7.00	7.00	7.00	7.00
8.00	8.00	8.00	8.00	8.05	8.00	8.00	8.00	8.00
10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	9.95
12.00	12.00	12.00	12.07	12.00	12.00	12.00	12.00	11.97
24.00	24.00	24.00	24.00	24.00	23.92	24.08	24.00	24.00

Table 9

Table 10

Vital Statistics of Subjects

Patient #	Race	Age	Height (cm)	Weight (kg)
01	C	21	184.0	76.5
02	C	27	177.0	71.0
03	C	27	179.4	76.0
04	C	27	173.1	63.1
05	B	35	187.0	66.8
06	C	33	178.2	77.0
07	C	27	180.0	77.6
08	B	23	192.0	82.5
09	B	29	182.0	78.0
10	B	23	176.5	71.0
11	C	24	183.8	66.4
12	C	29	185.6	83.2
13	C	20	203.5	83.4
14	B	28	182.8	84.4
15	B	29	197.0	100.3
16	B	27	189.7	63.9
AVERAGE ± S.D.		27 ± 4	184.5 ± 8.0	76.3 ± 9.5
RANGE		20-35	173.1-203.5	63.1-100.3

Table 11

Summary of Adverse Events

Subject	Adverse Event	Relationship to Study Drug*	Formulation**
1	Hematocrit decreased >5% over the course of the study	Possible	STD
2	Hematocrit decreased >5% over the course of study	Possible	STD
	Slightly elevated alanine aminotransferase at discharge, normal 9 days later	Possible	
	Brief ringing in ears after first oral dose with food	Possible	
3	Localized rash on forehead appearing during multiple-dose portion, present in past, resolved despite continued drug consumption	Remote	SR
	Hematocrit decreased >5% over the course of the study	Possible	
4	Hematocrit decreased >5% over the course of the study	Possible	SR
5	Flatulence, abdominal cramps, heartburn starting 10 minutes after intravenous infusion began, resolved after 4 hours even while infusion continued; same symptoms with single-dose given fasting, starting 10 minutes after dosing, resolving after 4 1/2 hours	Probable	STD
	Hematocrit decreased >5% over the course of the study	Possible	

*For definition of terms used in this colum, see Appendix G

**STD = Standard Tablet
SR = Sustained-release Tablet

Table 11 (cont'd)

Summary of Adverse Events

Subject	Adverse Event	Relationship to Study Drug*	Formulation**
6	Hematocrit decreased >5% over the course of the study	Possible	STD
	Broke tooth while eating	Remote	
7	Headache after starting multiple-dose portion	Possible	SR
	Nausea and diarrhea after starting multiple-dose portion	Possible	
8	Brief "rumbling in stomach" on first evening of multiple-dose portion, starting about 15 minutes after second dose.	Possible	SR
	Hematocrit decreased >5% over the course of the study	Possible	
9	Flatus starting about one hour after intravenous infusion began, resolved about 10 hours after infusion ended	Possible	STD
	Headache during first evening of multiple-dose portion, beginning shortly after receiving second dose of the day, resolved six hours later after receiving acetaminophen	Possible	
10	Hematocrit decreased >5% over the course of the study	Possible	STD

*For definition of terms used in this colum, see Appendix G

**STD = Standard Tablet
SR = Sustained-release Tablet

Table 11 (cont'd)

Summary of Adverse Events

Subject	Adverse Event	Relationship to Study Drug*	Formulation**
14	Hematocrit decreased >5% over the course of the study	Possible	STD
	Aminotransferases slightly elevated at discharge	Possible	
15	Hematocrit decreased >5% over the course of the study	Possible	SR
	Nausea starting 20 minutes after intravenous infusion began, resolved after one hour even with continuation of infusion; also had nausea starting 50 minutes after single-dose given fasting, lasted about 40 minutes	Probable	
	Alanine aminotransferase slightly elevated at discharge	Possible	
16	Hematocrit decreased >5% over the course of the study	Possible	SR

* For definition of terms used in this column, see Appendix G

** STD = Standard Tablet
SR = Sustained-release Tablet

Table 12

Pyridostigmine Base Concentrations (ng/ml) After
Intravenous Infusion in Subjects Receiving the Standard Tablet

SCH_INT (hours)	Subject 1	2	5	6	9	10	13	14	#	MEAN	SD	CV (%)	MAX	MIN
0.00	*	*	*	*	*	*	*	*	0					
0.125	3.87	5.41	2.32	*	8.54	5.34	3.66	2.17	7	4.47	2.20	49.27	8.54	2.17
0.25	10.40	7.55	11.50	3.42	13.80	7.28	10.20	3.57	8	8.47	3.70	43.77	13.80	3.42
0.375	11.50	11.30	12.20	4.88	8.90	12.70	11.00	3.68	8	9.52	3.43	36.08	12.70	3.68
0.50	13.60	12.40	14.70	6.16	24.30	10.20	11.70	6.07	8	12.39	5.77	46.53	24.30	6.07
1.00	15.80	15.00	15.50	7.95	22.70	10.50	14.50	10.60	8	14.07	4.52	32.10	22.70	7.95
1.50	16.50	15.20	18.50	10.70	21.70	12.60	18.20	11.20	8	15.58	3.89	24.95	21.70	10.70
2.00	15.40	18.10	17.90	12.10	21.60	19.00	18.10	12.90	8	16.89	3.20	18.95	21.60	12.10
3.00	18.60	18.00	17.80	14.30	20.10	17.00	21.80	16.40	8	18.00	2.28	12.69	21.80	14.30
4.00	26.20	17.80	19.00	14.90	20.00	17.40	22.90	14.90	8	19.14	3.88	20.29	26.20	14.90
5.00	19.00	15.70	17.70	16.50	25.10	18.00	23.00	14.30	8	18.66	3.67	19.66	25.10	14.30
6.00	NS	18.20	19.30	14.90	22.80	16.80	22.20	13.90	7	18.30	3.41	18.62	22.80	13.90
6.125	12.00	11.20	14.70	14.40	18.70	9.84	16.30	13.80	8	13.67	2.86	20.59	18.70	9.84
6.25	10.30	8.62	14.50	13.10	13.60	8.87	16.60	10.50	8	12.01	2.87	23.87	16.60	8.62
6.375	8.77	6.36	10.90	10.50	14.70	7.84	10.20	9.42	8	9.84	2.47	25.12	14.70	6.36
6.50	7.36	6.52	9.36	10.70	11.90	6.44	9.62	8.51	8	8.80	1.97	22.37	11.90	6.44
7.00	5.30	4.42	10.60	5.79	8.05	5.45	5.53	4.53	8	6.21	2.10	33.75	10.60	4.42
7.50	3.79	3.29	6.10	6.03	8.05	*	3.84	6.21	7	5.33	1.73	32.50	8.05	3.29
8.00	4.00	1.95	5.60	5.26	5.09	3.65	2.27	3.87	8	3.96	1.34	33.95	5.60	1.95
9.00	2.09	*	5.03	3.68	3.30	*	*	2.06	5	3.23	1.24	38.26	5.03	2.06
10.00	*	*	2.91	3.21	3.06	*	2.77	*	4	2.99	0.19	6.35	3.21	2.77
12.00	*	*	BC	*	*	*	*	*	0					
14.00	NS	NS	1.89	*	*	*	*	*	1	1.89			1.89	1.89
24.00	NS	*	*	NS	*	*	NS	NS	0					

* Concentration below limit of detectability (1.6 ng/ml)

NS = no sample

BC = bad chromatogram (i.e., technical difficulties prevented assay)

Comments on the protocol PK-PD of pyridostigmine

By Moshe J. Shmuklarsky **July 23 1992**

1. Please number pages, figures, table etc.
2. There is no discussion of kinetic-dynamic results from prior studies in the introduction, why?
3. The clinical part of the report is, overall, excellent.
4. Graphic presentation of the study design would make the design more apparent since there are several components to the design i.e. iv vs. oral; standard vs. sustained; fasting vs. food, single dose vs. multiple doses.
5. Standard breakfast should be described: total calories, protein, carbohydrates, fat etc.
6. The kinetic equation to which the data was fitted should be explicitly expressed with definition of all terms in the appendix. Attaching the computer output of PCNONLIN is not a sufficient substitute.
7. Why no attempt is being made to develop kinetic-dynamic model is not clear. Why?
8. Why AUCs were not calculated to infinity and why Cmax and Tmax are not provided? They are also measures of bioavailability.
9. The report should focus on noncompartmental analysis (i.e. trapezoidal AUC to infinity. Any results from compartmental analysis should be compared to the noncompartmental results?
10. AUCs were determined to last dose for different time periods (i.e 8 hr. and 12 hr.) rather than to infinity. Comparing these is like comparing apples and oranges. In addition, Were all AUCs normalized to unit dose or same dosage, the text is not very clear about this. If not the comparison of the AUCs needs to be normalized to the same dose.
11. All tables need to have units of measurements.
12. It would have been very helpfull to see graphic presentation of blood levels and cholinesterase inhibition superposed in the same graph.
14. Aslo would have been desirable to present graphically time-concentration profiles of, iv vs. PO standard, PO standard vs. sustained, single dose vs. multiple dose etc.

SGRD-UWM-C 24 June 1992

MEMORANDUM FOR THE RECORD

SUBJECT: Review Comments for Draft Final Report of "Safety, Tolerance ... Oral Pyridostigmine Pharmacokinetics" - Date of Review, 23 June 1992

1. Overall, the report was better organized and had a better background section than previous reports submitted as drafts. The Introduction was appropriate except for the failure of the authors to distinguish between prophylactic treatment and pretreatment roles of pyridostigmine (a pretreatment).

2. Some of the references to previous reports are inaccurate and previous drug preparation assays are not furnished to the appropriate report.

3. Drug side effects of pyridostigmine in patients are well annotated and documented at the end of the report.

4. The final conclusions of the report are valid but the objectives of the study, which were somewhat ambitious, were not completely met.

5. Specific (minor) criticisms of the report would include the items listed below.

a. There should be a complete bottle and lot number reference with a reference for the preparations used cited on page 13.

b. What is the assay of the i.v. pyridostigmine preparation; how were the doses on page 43 quantitated?

c. Page 15. Women are excluded in this protocol. Is there any explanation for this?

d. Page 23. Are strict pairwise comparisons appropriate in a study where three treatments are given?

6. An additional criticism that is a personal observation but, nonetheless, important in the overall review of this report is that for a pharmacokinetic and pharmacodynamic study there is a marked absence of time concentration plots for visual evaluation (which is referred to frequently in the text) by the reviewer. At the very minimum, a table listing the simultaneous cholinesterase inhibition values and plasma pyridostigmine values should be listed (and probably plotted, since the data are available). A tabulation and direct comparison of model-dependent and model-independent pharmacokinetic parameters should be included.

THOMAS G. BREWER
COL, MC
Department of Pharmacology
Division of Experimental Therapeutics

Table 20 describing iv curve fits for half of the volunteers was missing from my copy of the report.

Multiple dosing is poorly described on p 17, the methods do not describe the time between dosing

appendix B states that the sustained release formulation is given every 12 hours

Is the "standard" tablet given every 8 hours??

The analytical methods for the analysis of pyridostigmine are properly presented. The analytical analysis of pyridostigmine was carried out on contract by E. Lin as indicated on page 20 and the method carried out as referenced by #21.

Page 21 indicates "pyridostigmine concentrations of the subjects of this study are listed in that report"
NOT TRUE! THE DATA ARE SHOWN IN REPORT Pyr/P 89-8A which was sent to their lab In December 1990.
the complete reference: Lin, E.T., L. Z. Benet, R.A. Upton and W.L. Gee,. Routine Analysis of Pyridostigmine Samples for the Protocol Titled "Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Intravenous Pyridostigmine and Oral Doses of Standard and Sustained Release Pyridostigmine in Healthy Men and the Influence of Food on Oral Pyridostigmine Pharmacokinetics."
Analysis Report Pyr/P 89-8A Contract No. DAMD17-86-C-6150, 13 November 1990.

All of the data was properly transferred from the Lin report and incorporated into the data analysis section for the pharmacokinetic evaluation.

The analytical methods for the determination of erythrocyte acetylcholinesterase activity are properly presented in appendix D even though the entire SOP for the procedure is not included. Whole blood is centrifuged and the erythrocytes are then "picked up" from the bottom of the centrifuge tube. The activity is compared to an electric eel AChE standard

d. The clinical data demonstrated only limited adverse effects; side effects are presented as raw data in FAX format. Some possible relationship to pyridostigmine dosing and cholinesterase inhibition is suggested on page 27 and 28.

The fact that the adverse reactions occurred during the intravenous and the oral dosing in the same two volunteers is significant and should be discussed in the report.. This indicates that the adverse reaction is not due to a "local" action of the drug at the GI level, but is probably related to the systemic cholinesterase inhibition. It has often been assumed that pyridostigmine would stimulate gut motility directly and thereby enhance its own elimination.

Table 13

Pyridostigmine Base Concentrations (ng/ml)
after Intravenous Infusion in Subjects Receiving
the Sustained-Release Tablet

SCH_INT (hours)	Subject 3	4	7	8	11	12	15	16	#	MEAN	SD	CV (%)	MAX	MIN
0.00	*	*	*	*	*	*	*	*	0					
0.125	5.54	3.66	9.53	*	6.40	4.26	*	5.59	6	5.83	2.07	35.43	9.53	3.66
0.25	6.95	6.70	15.70	1.97	9.89	9.87	4.24	8.10	8	7.93	4.13	52.15	15.70	1.97
0.375	11.50	6.67	16.40	3.93	12.70	7.67	6.99	9.90	8	9.47	3.98	41.99	16.40	3.93
0.50	12.70	9.31	13.90	4.05	12.20	11.70	8.47	13.60	8	10.74	3.32	30.88	13.90	4.05
1.00	16.90	9.83	12.80	5.21	16.20	16.00	12.60	13.80	8	12.92	3.89	30.12	16.90	5.21
1.50	15.30	12.80	24.30	9.09	26.80	16.00	12.20	13.50	8	16.25	6.14	37.81	26.80	9.09
2.00	20.50	16.00	23.20	11.60	27.00	17.00	12.30	15.20	8	17.85	5.35	29.98	27.00	11.60
3.00	19.90	16.70	27.40	17.50	25.80	15.50	13.70	17.50	8	19.25	4.89	25.39	27.40	13.70
4.00	18.40	16.40	17.50	21.70	30.60	17.90	14.80	15.40	8	19.09	5.11	26.78	30.60	14.80
5.00	21.00	18.00	28.00	20.70	28.30	19.70	12.00	19.20	8	20.86	5.31	25.44	28.30	12.00
6.00	24.30	15.00	15.80	20.00	22.30	20.10	19.60	20.70	8	19.73	3.08	15.62	24.30	15.00
6.125	20.90	13.00	15.80	15.70	19.50	12.70	10.10	15.30	8	15.38	3.55	23.12	20.90	10.10
6.25	16.00	12.00	15.10	16.40	15.60	9.89	8.91	10.90	8	13.10	3.01	22.98	16.40	8.91
6.375	13.60	9.53	14.80	15.30	9.44	10.10	7.87	11.80	8	11.56	2.75	23.83	15.30	7.87
6.50	11.70	7.52	9.17	14.20	12.30	8.66	6.58	4.53	8	9.33	3.22	34.49	14.20	4.53
7.00	7.30	5.69	7.95	11.20	11.80	6.06	4.75	4.97	8	7.47	2.72	36.41	11.80	4.75
7.50	NS	NS	5.24	8.97	8.09	3.37	3.10	4.07	6	5.47	2.50	45.61	8.97	3.10
8.00	3.73	2.93	4.01	6.02	4.18	3.35	2.33	*	7	3.79	1.17	30.90	6.02	2.33
9.00	2.64	2.25	3.93	3.66	2.83	2.24	*	*	6	2.93	0.72	24.49	3.93	2.24
10.00	1.85	*	*	3.10	*	*	*	*	2	2.48	0.88	35.71	3.10	1.85
12.00	*	*	*	2.29	*	2.14	*	*	2	2.22	0.11	4.79	2.29	2.14
14.00	*	*	*	*	NS	NS	NS	NS	0					
24.00	NS	NS	*	*	*	*	NS	NS	0					

* Concentration below limit of detectability (1.6 ng/ml)

NS = no sample

Table 15

Pyridostigmine Base Concentrations (ng/ml) in Subjects
after Receiving One Standard Tablet While Fasting

SCH_INT (hours)	Subject 1	2	5	6	9	10	13	14	#	MEAN	SD	CV (%)	MAX	MIN
0.00	*	*	*	*	*	*	*	*	0					
0.25	*	*	1.74	*	*	3.00	*	*	2	2.37	0.89	37.59	3.00	1.74
0.50	11.60	*	19.10	4.34	*	8.63	*	4.36	5	9.61	6.13	63.82	19.10	4.34
0.75	22.60	13.10	22.10	8.63	4.78	9.01	13.60	10.20	8	13.00	6.39	49.14	22.60	4.78
1.00	26.30	16.70	27.50	8.76	16.00	12.00	20.70	14.80	8	17.85	6.59	36.90	27.50	8.76
1.33	23.20	16.50	24.90	12.40	24.00	NS	32.10	17.00	7	21.44	6.60	30.76	32.10	12.40
1.67	22.60	14.30	23.50	17.80	29.80	9.04	32.20	16.40	8	20.71	7.84	37.87	32.20	9.04
2.00	21.00	13.30	25.20	13.40	22.30	7.66	30.80	13.30	8	18.37	7.69	41.88	30.80	7.66
2.50	23.40	11.40	26.20	14.30	20.20	6.46	34.50	10.50	8	18.37	9.40	51.19	34.50	6.46
3.00	18.90	12.00	26.00	11.80	27.20	7.39	36.30	7.08	8	18.33	10.62	57.93	36.30	7.08
3.50	20.20	11.00	19.60	12.10	23.30	6.07	27.90	7.52	8	15.96	7.89	49.46	27.90	6.07
4.00	16.20	7.30	20.30	11.90	28.40	7.83	22.90	8.07	8	15.36	7.92	51.54	28.40	7.30
5.00	12.10	6.58	15.40	8.11	18.00	6.75	18.20	7.36	8	11.56	5.04	43.62	18.20	6.58
6.00	7.52	4.50	9.16	6.12	12.00	3.16	12.20	6.45	8	7.64	3.29	43.07	12.20	3.16
7.00	5.35	3.51	8.44	4.43	6.65	3.64	9.83	4.25	8	5.76	2.34	40.60	9.83	3.51
8.00	3.74	2.30	6.45	4.01	6.82	*	7.27	*	6	5.10	2.02	39.59	7.27	2.30
10.00	2.46	1.84	4.69	2.94	1.78	*	6.15	*	6	3.31	1.75	52.90	6.15	1.78
12.00	2.30	*	3.39	2.23	*	*	4.64	*	4	3.14	1.13	36.06	4.64	2.23
14.00	2.22	NS	1.79	*	*	*	3.37	*	3	2.46	0.82	33.21	3.37	1.79
24.00	*	*	*	*	*	*	2.62	*	1	2.62			2.62	2.62

* Concentration below limit of detectability (1.6 ng/ml)

NS = no sample

Table 15

Pyridostigmine Base Concentrations (ng/ml) in Subjects
after Receiving One Sustained-Release Tablet While Fasting

SCH_INT (hours)	Subject 3	4	7	8	11	12	15	16	#	MEAN	SD	CV (%)	MAX	MIN
0.00	*	*	*	*	*	*	*	*	0					
0.25	7.72	2.91	3.57	*	2.55	*	*	1.94	5	3.74	2.30	61.61	7.72	1.94
0.50	24.20	6.67	4.63	2.74	5.90	7.03	2.95	5.10	8	7.40	6.97	94.10	24.20	2.74
0.75	25.90	11.20	14.10	7.54	6.32	9.99	8.20	12.30	8	11.94	6.20	51.90	25.90	6.32
1.00	30.70	20.70	11.30	13.40	7.92	12.00	11.10	18.30	8	15.68	7.34	46.80	30.70	7.92
1.33	36.90	44.40	11.20	18.30	10.80	12.90	26.40	18.80	8	22.46	12.48	55.56	44.40	10.80
1.67	28.60	69.20	9.15	19.00	12.30	18.50	45.60	30.20	8	29.07	19.92	68.51	69.20	9.15
2.00	26.10	55.20	9.33	18.50	15.00	27.20	27.90	25.70	8	25.62	13.69	53.44	55.20	9.33
2.50	21.50	36.00	16.40	47.50	15.70	20.00	21.70	20.70	8	24.94	11.05	44.30	47.50	15.70
3.00	19.90	33.30	17.30	35.50	14.20	23.90	20.20	17.80	8	22.76	7.72	33.91	35.50	14.20
3.50	16.10	27.60	15.60	29.50	11.00	22.90	18.40	17.30	8	19.80	6.34	32.03	29.50	11.00
4.00	18.00	26.00	28.50	26.10	10.50	17.70	14.50	16.70	8	19.75	6.39	32.34	28.50	10.50
5.00	13.50	20.20	19.10	18.10	10.50	11.50	9.49	10.50	8	14.11	4.35	30.83	20.20	9.49
6.00	9.53	18.10	8.74	14.20	6.88	9.51	6.49	8.02	8	10.18	3.98	39.10	18.10	6.49
7.00	7.05	11.20	5.18	15.00	6.51	5.92	4.14	4.37	8	7.42	3.78	50.94	15.00	4.14
8.00	6.33	10.60	2.89	8.79	6.99	4.93	5.18	3.96	8	6.21	2.54	40.99	10.60	2.89
10.00	4.56	5.09	1.78	5.72	*	2.76	2.60	3.37	7	3.70	1.45	39.26	5.72	1.78
12.00	2.19	3.78	*	4.88	3.61	2.21	2.39	1.86	7	2.99	1.12	37.33	4.88	1.86
14.00	*	2.70	*	2.23	*	2.01	2.29	1.80	5	2.21	0.34	15.29	2.70	1.80
24.00	*	*	*	*	*	*	*	*	0					

* Concentration below limit of detectability (1.6 ng/ml)

Table 16

Pyridostigmine Base Concentrations (ng/ml) in Subjects
after Receiving One Standard Tablet with Food -- First Dose

SCH_INT (hours)	Subject 1	2	5	6	9	10	13	14	#	MEAN	SD	CV (%)	MAX	MIN
0.00	*	*	*	*	*	*	*	*	0					
0.25	4.36	3.13	*	*	*	*	*	7.19	3	4.89	2.08	42.55	7.19	3.13
0.50	6.55	7.38	2.66	2.61	*	*	8.95	24.90	6	8.84	8.27	93.58	24.90	2.61
0.75	10.50	11.30	3.43	3.59	5.17	10.10	16.60	19.10	8	9.97	5.81	58.20	19.10	3.43
1.00	13.30	13.20	6.49	5.51	7.08	11.30	15.20	25.40	8	12.19	6.43	52.81	25.40	5.51
1.33	13.40	12.90	6.82	9.84	7.35	12.10	14.20	17.20	8	11.73	3.53	30.10	17.20	6.82
1.67	13.10	12.30	8.44	10.10	8.83	12.90	14.90	13.80	8	11.80	2.38	20.20	14.90	8.44
2.00	14.10	11.90	9.66	10.00	9.27	14.00	14.50	13.00	8	12.05	2.16	17.90	14.50	9.27
2.50	15.30	10.80	10.10	9.77	11.60	13.80	14.70	8.35	8	11.80	2.52	21.39	15.30	8.35
3.00	14.90	10.50	10.90	13.50	13.10	15.00	14.60	7.25	8	12.47	2.72	21.84	15.00	7.25
3.50	12.80	10.30	10.20	14.60	14.10	14.80	14.40	6.12	8	12.17	3.07	25.22	14.80	6.12
4.00	12.90	9.83	11.60	15.30	17.30	9.10	14.80	5.21	8	12.01	3.91	32.57	17.30	5.21
5.00	11.80	8.76	9.81	7.41	12.50	9.48	14.80	3.51	8	9.76	3.44	35.23	14.80	3.51
6.00	6.42	7.06	5.70	6.67	9.36	4.32	9.51	4.04	8	6.64	2.03	30.62	9.51	4.04
7.00	4.13	3.91	5.65	5.06	6.02	3.73	7.97	2.96	8	4.93	1.60	32.50	7.97	2.96
8.00	4.62	3.64	4.98	4.81	5.09	3.03	6.82	*	7	4.71	1.20	25.48	6.82	3.03
							10.60	6.53	2	8.57	2.88	33.60	10.60	6.53

* Concentration below limit of detectability (1.6 ng/ml)

Table 17

Pyridostigmine Base Concentrations (ng/ml) in Subjects
after Receiving One Sustained-Release Tablet with Food -- First Dose

SCH_INT (hours)	Subject 3	4	7	8	11	12	15	16	#	MEAN	SD	CV (%)	MAX	MIN
0.00	*	*	*	*	*	*	*	*	0					
0.25	*	1.75	*	*	*	2.01	1.93	*	3	1.90	0.13	7.02	2.01	1.75
0.50	5.18	4.24	1.72	7.33	*	7.23	7.95	1.81	7	5.07	2.60	51.36	7.95	1.72
0.75	10.50	8.15	2.93	8.85	3.57	8.90	15.00	4.93	8	7.85	3.99	50.79	15.00	2.93
1.00	15.30	10.00	5.10	7.39	5.05	12.00	15.90	6.64	8	9.67	4.35	45.01	15.90	5.05
1.33	16.30	15.20	3.14	19.90	10.50	15.70	16.90	8.54	8	13.27	5.46	41.13	19.90	3.14
1.67	17.70	18.40	4.90	18.10	10.90	17.90	20.10	12.70	8	15.09	5.17	34.25	20.10	4.90
2.00	15.70	18.60	4.01	28.80	16.10	21.10	21.50	15.40	8	17.65	7.06	40.02	28.80	4.01
2.50	13.00	16.90	8.30	41.30	18.50	27.10	19.00	23.10	8	20.90	10.04	48.05	41.30	8.30
3.00	12.40	26.40	10.30	47.90	29.60	31.40	19.00	27.90	8	25.61	11.98	46.78	47.90	10.30
3.50	12.40	28.60	11.50	43.50	35.10	34.30	22.60	28.50	8	27.06	11.14	41.16	43.50	11.50
4.00	10.50	26.60	8.87	47.70	37.00	35.50	23.20	34.70	8	28.01	13.46	48.04	47.70	8.87
5.00	8.84	25.90	11.60	47.30	38.10	40.00	22.90	43.10	8	29.72	14.59	49.08	47.30	8.84
6.00	6.49	16.10	4.44	37.40	31.00	26.10	14.00	25.70	8	20.15	11.77	58.38	37.40	4.44
7.00	6.85	14.60	3.79	27.10	23.10	21.20	10.80	15.20	8	15.33	8.10	52.85	27.10	3.79
8.00	5.79	12.30	1.67	19.20	26.10	14.90	8.57	11.30	8	12.48	7.70	61.69	26.10	1.67
10.00	4.89	7.35	*	6.29	14.30	9.60	6.55	7.72	7	8.10	3.09	38.18	14.30	4.89
12.00	3.48	5.11	*	4.11	8.34	6.48	3.95	4.97	7	5.21	1.70	32.59	8.34	3.48

* Concentration below limit of detectability (1.6 ng/ml)

Table 18

Pyridostigmine Base Concentrations (ng/ml) in Subjects
after Receiving One Standard Tablet on Day 2 of
Multiple-Dose Portion

SCH_INT (hours)	Subject 1	2	5	6	9	10	13	14	#	MEAN	SD	CV (%)	MAX	MIN
0.00	11.70	5.50	9.94	2.73	*	9.63	11.40	4.58	7	7.93	3.59	45.30	11.70	2.73
0.25	14.60	7.68	9.94	4.43	7.71	9.63	10.30	6.26	8	8.82	3.07	34.78	14.60	4.43
0.50	20.70	10.20	12.80	5.73	15.90	13.00	14.30	13.70	8	13.29	4.31	32.42	20.70	5.73
0.75	24.20	11.60	13.30	6.17	23.50	17.40	14.50	18.50	8	16.15	6.06	37.53	24.20	6.17
1.00	20.50	14.20	13.60	6.98	23.30	16.80	19.00	20.30	8	16.84	5.16	30.66	23.30	6.98
1.33	19.80	12.40	12.90	9.51	21.50	21.00	23.30	18.80	8	17.40	5.07	29.13	23.30	9.51
1.67	18.60	12.80	14.50	15.60	23.30	19.20	22.90	21.40	8	18.54	3.93	21.21	23.30	12.80
2.00	16.70	12.50	15.30	14.10	19.60	14.10	23.80	22.10	8	17.28	4.11	23.81	23.80	12.50
2.50	14.80	11.30	14.70	14.50	17.40	14.60	25.80	20.50	8	16.70	4.53	27.14	25.80	11.30
3.00	13.50	12.00	16.10	17.50	14.30	17.60	25.10	17.00	8	16.64	3.97	23.88	25.10	12.00
3.50	13.50	12.10	17.00	15.10	12.80	15.40	23.40	15.40	8	15.59	3.54	22.70	23.40	12.10
4.00	13.00	9.81	15.80	19.80	12.10	12.40	22.40	15.90	8	15.15	4.23	27.91	22.40	9.81
5.00	12.70	9.75	16.00	17.20	9.31	10.30	16.20	8.48	8	12.49	3.52	28.19	17.20	8.48
6.00	9.37	5.93	12.50	10.90	5.70	9.16	14.60	7.00	8	9.40	3.17	33.77	14.60	5.70
7.00	7.33	5.51	10.60	7.31	4.08	8.16	13.00	5.76	8	7.72	2.89	37.50	13.00	4.08
8.00	5.48	4.21	7.97	6.32	3.20	5.34	9.62	5.88	8	6.00	2.03	33.82	9.62	3.20
24.00	9.55	4.26	8.64	7.41	3.06	11.90	11.40	7.96	8	8.02	3.12	38.94	11.90	3.06

* Concentration below limit of detectability (1.6 ng/ml)

Table 18

Pyridostigmine Base Concentrations (ng/ml) in Subjects after Receiving One Sustained-Release Tablet on Day 2 of Multiple-Dose Portion

SCH_INT (hours)	Subject 3	4	7	8	11	12	15	16	#	MEAN	SD	CV (%)	MAX	MIN
0.00	21.80	10.70	14.20	9.61	8.29	17.80	12.80	11.30	8	13.31	4.52	33.96	21.80	8.29
0.25	21.70	12.60	13.90	14.40	5.89	18.50	14.00	11.80	8	14.10	4.66	33.07	21.70	5.89
0.50	22.50	13.80	17.80	17.10	NS	25.40	23.00	15.70	7	19.33	4.31	22.30	25.40	13.80
0.75	23.90	15.00	23.40	18.60	10.60	26.80	22.10	13.50	8	19.24	5.74	29.82	26.80	10.60
1.00	28.70	15.10	23.30	26.50	17.50	27.20	22.90	14.20	8	21.93	5.65	25.76	28.70	14.20
1.33	29.70	17.50	31.20	27.90	25.30	29.70	22.60	11.60	8	24.44	6.87	28.10	31.20	11.60
1.67	32.10	18.10	31.10	27.10	22.80	30.90	21.90	13.90	8	24.74	6.67	26.97	32.10	13.90
2.00	31.80	18.00	30.80	23.30	22.50	31.90	20.60	14.10	8	24.13	6.74	27.92	31.90	14.10
2.50	40.40	20.30	33.80	20.20	30.00	34.70	22.10	10.80	8	26.54	9.78	36.87	40.40	10.80
3.00	44.60	19.10	32.30	18.30	31.60	41.20	20.60	11.40	8	27.39	11.84	43.24	44.60	11.40
3.50	37.30	21.00	33.40	16.70	34.50	42.40	20.40	9.05	8	26.84	11.64	43.35	42.40	9.05
4.00	47.00	23.80	36.20	14.40	40.30	40.20	20.50	7.62	8	28.75	14.13	49.15	47.00	7.62
5.00	37.80	24.00	42.90	13.20	41.60	37.90	16.00	6.29	8	27.46	14.39	52.39	42.90	6.29
6.00	29.10	19.90	24.30	11.80	31.40	24.40	10.50	5.10	8	19.56	9.48	48.44	31.40	5.10
7.00	25.00	16.20	18.30	8.91	23.90	16.20	6.99	3.88	8	14.92	7.73	51.77	25.00	3.88
8.00	20.90	14.00	14.60	7.72	22.80	13.90	7.28	3.59	8	13.10	6.67	50.96	22.80	3.59
10.00	18.30	9.11	7.96	5.90	16.30	8.19	5.97	3.64	8	9.42	5.18	54.95	18.30	3.64
12.00	14.10	7.98	7.52	5.72	11.20	6.76	3.10	2.48	8	7.36	3.88	52.79	14.10	2.48
24.00	24.20	9.13	5.50	20.30	25.80	9.20	14.30	7.43	8	14.48	7.96	54.95	25.80	5.50

NS = no sample

Table 24

IV Curve fits - 2 Compartments
Sustained Release

	3	4	7	8	11	12	15	16	MEAN	SD	CV (%)	MAX	MIN
V	16.400	17.500	5.450	44.730	9.130	17.240	27.710	14.500	19.083	12.255	64.219	27.710	5.450
SEM	2.120	4.380	3.710	5891.940	3.750	1.870	5.000	2.590	739.420	2081.933	281.563	5891.940	1.870
K10	1.710	2.310	5.560	0.800	3.000	1.960	1.650	1.750	2.343	1.441	61.529	5.560	0.800
SEM	0.230	0.580	3.810	104.740	1.240	0.300	1.050	6.090	14.755	36.417	246.813	104.740	0.230
K12	0.780	2.560	5.710	23.510	3.220	0.860	0.620	1.690	4.869	7.720	158.564	23.510	0.620
SEM	0.400	1.830	6.340	12148.850	3.260	0.180	0.400	5.220	1520.810	4294.377	282.374	12178.850	0.400
K21	0.670	1.640	1.170	34.410	1.540	0.230	0.250	0.130	5.005	11.896	237.689	34.410	0.130
SEM	0.310	0.690	0.680	6640.440	0.880	0.120	0.870	0.680	830.584	2347.536	282.637	6640.440	0.120
AUC	142.500	103.430	147.970	118.740	162.300	131.310	98.960	171.790	134.625	26.446	19.644	171.790	98.960
SEM	4.320	2.840	9.460	5.850	7.210	11.150	50.240	576.460	83.441	199.806	239.457	576.460	2.840
ALPHA	2.740	5.860	11.900	58.240	7.110	2.890	2.340	3.500	11.823	19.026	160.927	58.240	2.340
SEM	0.780	2.920	10.400	18893.090	5.090	0.430	1.000	1.190	2364.363	6678.615	282.470	18893.090	0.430
BETA	0.420	0.650	0.550	0.470	0.650	0.160	0.180	0.063	0.393	0.231	58.674	0.650	0.063
SEM	0.130	0.110	0.200	0.140	0.170	0.090	0.650	0.540	0.254	0.215	84.862	0.650	0.090
ALPHA-HL	0.250	0.120	0.058	0.012	0.097	0.240	0.300	0.200	0.160	0.103	64.227	0.300	0.012
SEM	0.070	0.060	0.051	3.860	0.070	0.040	0.130	0.070	0.544	1.340	246.413	3.860	0.040
BETA-HL	1.660	1.070	1.270	1.480	1.070	4.440	3.880	11.040	3.239	3.414	105.407	11.040	1.070
SEM	0.520	0.180	0.470	0.140	0.280	2.420	14.220	95.210	14.180	33.091	233.363	95.210	0.140
A	218.120	193.080	777.990	38.950	419.070	250.590	157.140	295.820	293.845	224.085	76.260	777.990	38.950
SEM	25.140	52.820	548.980	12415.890	182.710	26.530	21.230	46.710	1665.001	4347.709	261.123	12415.890	21.230
B	26.210	45.510	45.090	55.480	67.020	6.870	5.660	12.360	15.795	7.885	49.923	29.450	3.150
SEM	15.040	13.340	23.690	12.990	29.450	3.150	16.340	5.480	32.165	24.476	76.095	67.020	5.480

Table 22

PO Curve fits - Fasted and Fed, Using IV Constants
Standard Dose

	1	2	5	6	9	10	13	14	MEAN	SD	CV (%)	MAX	MIN
K01 STD-FAST	0.350	0.360	0.360	0.380	0.360	0.320	0.310	0.460	0.363	0.046	12.577	0.046	0.310
SEM	0.020	0.070	0.020	0.030	0.050	0.050	0.020	0.090	0.444	0.026	59.776	0.090	0.020
TLAGFA	0.380	0.400	0.230	0.360	0.720	0.200	0.610	0.300	0.400	0.180	45.059	0.072	0.200
SEM	0.030	0.080	0.010	0.040	0.020	0.020	0.030	0.080	0.039	0.027	69.572	0.090	0.010
FSTDFAST	0.190	0.130	0.220	0.150	0.180	0.100	0.280	0.150	0.175	0.056	32.180	0.280	0.100
SEM	0.010	0.010	0.010	0.010	0.020	0.010	0.010	0.010	0.011	0.004	31.427	0.020	0.010
AUC FAST	125.490	71.310	169.630	92.400	141.080	53.980	236.520	126.550	127.120	58.117	45.718	236.520	71.310
SEM	5.450	2.780	7.310	4.470	13.180	4.600	10.590	12.540	7.615	3.986	52.343	13.180	2.780
ABS-HLFA	1.950	1.940	1.940	1.820	1.920	2.180	2.260	1.500	1.939	0.229	11.834	2.260	1.500
SEM	0.130	0.120	0.130	0.160	0.280	0.310	0.160	0.300	0.199	0.083	41.615	0.310	0.120
K01 STD-FED	0.250	0.240	0.180	0.290	0.140	0.350	0.160	1.150	0.345	0.333	96.421	1.150	0.140
SEM	0.040	0.030	0.050	0.070	0.050	0.040	0.020	0.230	0.066	0.068	102.355	0.230	0.200
TLAGFE	0.380	0.340	0.620	0.600	0.610	0.600	0.150	0.100	0.425	0.215	50.621	0.620	0.100
SEM	0.060	0.060	0.070	0.090	0.090	0.070	0.090	0.060	0.074	0.014	19.090	0.090	0.060
FSTDFE	0.150	0.150	0.130	0.150	0.150	0.140	0.220	0.130	0.153	0.029	18.794	0.220	0.130
SEM	0.020	0.010	0.020	0.020	0.040	0.010	0.020	0.010	0.019	0.010	52.855	0.040	0.010
AUC FED	96.700	81.070	102.570	93.210	123.810	75.490	184.260	111.340	108.556	34.293	31.590	184.970	75.490
SEM	11.170	7.010	18.350	12.620	31.870	5.620	18.260	8.930	14.229	8.541	60.029	31.870	5.620
ABS-HLFE	2.820	2.850	3.890	2.430	5.010	1.990	4.290	0.600	2.985	1.397	46.789	5.010	0.600
SEM	0.510	0.390	1.050	0.570	1.810	0.250	0.640	0.120	0.668	0.540	80.830	1.810	0.120

Table 23

PO Curve fits - Fasted and Fed, Using IV Constants
Sustained Release

	3	4	7	8	11	12	15	16	MEAN	SD	CV (%)	MAX	MIN
K01 SR-FAST	0.380	0.470	0.260	0.350	0.160	0.260	0.580	0.420	0.360	0.133	37.060	0.470	0.160
SEM	0.030	0.100	0.070	0.050	0.030	0.050	0.100	0.070	0.063	0.028	44.233	0.100	0.030
TLAGFA	0.150	0.860	0.460	0.440	0.370	0.410	0.880	0.630	0.525	0.250	47.679	0.880	0.150
SEM	0.020	0.080	0.040	0.060	0.070	0.070	0.050	0.050	0.055	0.019	35.041	0.080	0.020
FSR/FAST	0.070	0.130	0.040	0.100	0.050	0.070	0.080	0.060	0.075	0.029	38.380	0.130	0.040
SEM	0.000	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.009	0.004	40.406	0.010	0.000
AUC FAST	149.610	203.900	94.190	182.780	112.980	127.880	118.970	153.350	142.958	36.901	25.81[illegible]	203.900	94.190
SEM	6.170	22.030	16.760	15.280	14.350	15.230	10.840	13.790	14.306	4.566	31.919	22.030	6.170
ABS-HLFA	1.820	1.470	2.690	1.990	4.220	2.680	1.200	1.640	2.214	0.970	43.811	4.220	1.200
SEM	0.130	0.300	0.700	0.280	0.800	0.480	0.200	0.250	0.393	0.244	62.091	0.800	0.130
K01 SR-FED	0.250	0.180	0.170	0.360	0.110	0.180	0.190	0.280	0.215	0.078	36.284	0.360	0.110
SEM	0.020	0.030	0.080	0.060	0.040	0.050	0.030	0.090	0.050	0.025	50.143	0.090	0.020
TLAGFE	0.350	0.810	0.680	0.900	1.230	1.170	0.350	1.860	0.919	0.502	54.609	1.860	0.350
SEM	0.040	0.050	0.090	0.090	0.090	0.140	0.060	0.110	0.084	0.033	39.311	0.140	0.040
FSR/FE	0.050	0.130	0.030	0.160	0.150	0.150	0.130	0.110	0.114	0.048	42.536	0.160	0.030
SEM	0.000	0.010	0.010	0.020	0.040	0.030	0.010	0.020	0.018	0.013	73.242	0.040	0.000
AUC FED	103.920	201.040	57.080	289.750	345.170	281.740	186.170	273.260	217.266	99.026	45.578	345.170	57.080
SEM	5.540	21.740	18.710	28.370	86.980	56.550	17.220	52.270	35.923	27.063	75.338	86.980	5.540
ABS-HLFE	2.750	3.870	3.970	1.920	6.370	3.940	3.640	2.490	3.619	1.351	37.330	6.370	1.920
SEM	0.240	0.630	1.870	0.320	2.220	1.200	0.510	0.790	0.973	0.731	75.165	2.220	0.240

Table 24

Bioavailability of Pyridostigmine Formulations
Determined From the Area Under the Pyridostigmine Base
Concentration-Time Curve

STANDARD TABLET

	SUBJECT 1	2	5	6	9	10	13	14	MEAN	STO DEV	%CV	MAX	MIN
AUC-IV	120.94	106.99	140.07	98.67	154.27	105.37	135.60	93.25	119.40	21.95	18.38	154.27	93.25
AUC-FASTING	126.27	66.83	157.14	82.26	138.40	45.93	187.21	59.14	107.90	51.47	47.70	187.21	45.93
AUC-FIRST OOSE FEO	79.72	67.38	60.48	65.93	75.41	67.66	95.27	59.75	71.45	11.77	16.48	95.27	59.75
AUC-STEAOY STATE FED	105.10	73.96	108.56	97.68	94.18	98.78	144.62	101.27	103.02	19.78	19.20	144.62	73.96
DOSE IV ug pyridostigmine base	3984	4272	4008	3936	4272	4248	4368	4440					
DOSE PO ug pyridostigmine base	20889	20889	20889	20889	20889	20889	20889	20889					
BIOAVAILABILITY-FASTING	0.20	0.13	0.22	0.16	0.18	0.09	0.29	0.13	0.17	0.06	35.50	0.29	0.09
BIOAVAILABILITY-FED-SS	0.17	0.14	0.15	0.19	0.12	0.19	0.22	0.23	0.18	0.04	21.64	0.23	0.12
FED BIOAVAIL/FASTING BIOAVAIL	0.83	1.11	0.69	1.19	0.68	2.15	0.77	1.71	1.14	0.53	46.76	2.15	0.68
ACCUMULATION-FEO-SS/1ST DOSE	1.32	1.10	1.79	1.48	1.25	1.46	1.52	1.69	1.45	0.23	15.76	1.79	1.10

SUSTAINEO-RELEASE TABLET

	SUBJECT 3	4	7	8	11	12	15	16	MEAN	STO DEV	%CV	MAX	MIN
AUC-IV	134.23	102.89	147.53	126.86	170.26	121.42	87.09	105.66	124.49	26.61	21.37	170.26	87.09
AUC-FASTING	150.74	226.60	104.70	186.57	96.92	122.50	122.63	118.57	141.15	44.63	31.62	226.60	96.92
AUC-FIRST DOSE FEO	97.16	175.68	49.37	292.36	253.28	236.38	157.45	204.25	183.24	81.18	44.30	292.36	49.37
AUC-STEAOY STATE FEO	332.32	190.53	264.01	150.70	290.72	273.02	152.82	82.22	217.04	85.74	39.50	332.32	82.22
OOSE IV ug pyridostigmine base	4008	4176	4488	4224	4440	4440	4512	4368					
OOSE PO ug pyridostigmine base	64056	64056	64056	64056	64056	64056	64056	64056					
BIOAVAILABILITY-FASTING	0.07	0.14	0.05	0.10	0.04	0.07	0.10	0.08	0.08	0.03	40.46	0.14	0.04
BIOAVAILABILITY-FED-SS	0.15	0.12	0.13	0.08	0.12	0.16	0.12	0.05	0.12	0.04	30.24	0.16	0.05
FED BIOAVAIL/FASTING BIOAVAIL	2.20	0.84	2.52	0.81	3.00	2.23	1.25	0.69	1.69	0.90	53.08	3.00	0.69
ACCUMULATION-FEO-SS/1ST OOSE	3.42	1.08	5.35	0.52	1.15	1.16	0.97	0.40	1.76	1.73	98.29	5.35	0.40

AUC are ng pyridostigmine base x hr x ml^{-1}

AUC-IV and AUC-Fasting are to last measured concentration. AUC-First Oose and AUC-Steady State Fed are for 8 hour interval in the case of the standard preparation and 12 hours for the sustained release preparation.

Table 25

Erythrocyte Acetylcholinesterase Inhibition After Intravenous Infusion in Subjects Receiving the Standard Tablet

Scheduled Interval (hours)	Subject 01	02	05	06	09	10	13	14	MEAN	SD	CV (%)	MAX	MIN
						Inhibition*							
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.01	0.01	282.8	0.04	0.00
0.25	0.04	0.03	0.04	0.00	0.08	0.07	0.07	0.00	0.04	0.03	75.2	0.08	0.00
0.50	0.10	0.09	0.10	0.06	0.19	0.19	0.12	0.04	0.11	0.06	49.5	0.19	0.04
1.00	0.20	0.19	0.20	0.15	0.22	0.23	0.20	0.13	0.19	0.03	17.7	0.23	0.13
1.50	0.25	0.28	0.25	0.20	0.28	0.28	0.29	0.23	0.26	0.03	12.6	0.29	0.20
2.00	0.26	0.29	0.29	0.26	0.27	0.31	0.32	0.26	0.28	0.02	8.5	0.31	0.26
3.00	0.29	0.29	0.35	0.31	0.38	0.32	0.35	0.30	0.33	0.03	10.4	0.38	0.29
4.00	0.33	0.32	0.37	0.31	0.36	0.33	0.39	0.33	0.34	0.03	8.0	0.39	0.31
5.00	0.28	0.27	0.38	0.32	0.37	0.33	0.36	0.27	0.32	0.04	13.6	0.38	0.27
6.00	0.30	0.26	0.35	0.31	0.36	0.30	0.40	0.35	0.33	0.04	13.5	0.40	0.26
6.25	0.22	0.21	0.26	0.27	0.34	0.24	0.34	0.33	0.28	0.05	19.0	0.34	0.21
6.50	0.20	0.17	0.26	0.26	0.28	0.21	0.29	0.23	0.24	0.04	17.0	0.29	0.17
7.00	0.16	0.14	0.20	0.19	0.20	0.14	0.20	0.17	0.17	0.02	14.1	0.20	0.14
7.50	0.11	0.08	0.16	0.14	0.17	0.12	0.16	0.14	0.14	0.03	22.6	0.17	0.08
8.00	0.04	0.05	0.15	0.12	0.15	0.06	0.13	0.11	0.10	0.04	43.2	0.15	0.04
9.00	0.03	0.02	0.15	0.08	0.10	0.08	0.08	0.08	0.07	0.04	54.2	0.15	0.02
10.00	0.06	0.04	0.10	0.06	0.09	0.04	0.06	0.03	0.06	0.03	42.6	0.09	0.03
12.00	0.00	0.00	0.07	0.09	0.05	0.04	0.03	0.00	0.03	0.03	96.8	0.09	0.00
14.00	ND	ND	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	162.5	0.01	0.00

ND = not done because inhibition already back to baseline

*Inhibition is expressed as the proportion of inhibited enzyme compared to baseline

Table 26

Erythrocyte Acetylcholinesterase Inhibition after Intravenous Infusion in Subjects Receiving the Sustained-Release Tablet

Scheduled Interval	Subject 03	04	07	08	11	12	15	16	MEAN	SD	CV (%)	MAX	MIN
				Inhibition*									
0.00	0.00	0.00	0.01	0.00	0.02	0.00	0.00	0.00	0.00	0.01	198.4	0.02	0.00
0.25	0.01	0.00	0.04	0.00	0.03	0.04	0.02	0.07	0.03	0.02	90.1	0.07	0.00
0.50	0.06	0.08	0.11	0.00	0.14	0.11	0.08	0.16	0.09	0.05	53.1	0.16	0.00
1.00	0.17	0.14	0.18	0.03	0.23	0.21	0.14	0.18	0.16	0.06	38.5	0.23	0.03
1.50	0.25	0.24	0.27	0.15	0.34	0.28	0.23	0.26	0.25	0.05	21.1	0.34	0.15
2.00	0.27	0.27	0.27	0.19	0.38	0.33	0.24	0.30	0.28	0.06	20.2	0.38	0.19
3.00	0.31	0.30	0.30	0.30	0.42	0.34	0.26	0.31	0.32	0.05	15.0	0.42	0.26
4.00	0.34	0.34	0.31	0.31	0.41	0.32	0.32	0.36	0.34	0.03	9.5	0.41	0.31
5.00	0.34	0.33	0.35	0.35	0.44	0.34	0.33	0.38	0.36	0.04	10.5	0.44	0.33
6.00	0.32	0.30	0.31	0.35	0.38	0.33	0.26	0.30	0.32	0.04	11.6	0.38	0.26
6.25	0.28	0.26	0.27	0.39	0.33	0.32	0.22	0.28	0.29	0.05	17.7	0.39	0.22
6.50	0.25	0.23	0.25	0.31	0.28	0.22	0.17	0.23	0.24	0.04	17.2	0.31	0.17
7.00	0.20	0.18	0.18	0.22	0.23	0.16	0.13	0.16	0.18	0.03	18.0	0.23	0.13
7.50			0.15	0.19	0.18	0.12	0.09	0.12	0.14	0.04	26.2	0.19	0.09
8.00	0.18	0.14	0.15	0.18	0.15	0.09	0.06	0.10	0.13	0.05	34.8	0.18	0.06
9.00	0.12	0.08	0.10	0.18	0.09	0.04	0.07	0.08	0.09	0.04	44.4	0.18	0.04
10.00	0.17	0.17	0.07	0.16	0.04	0.01	0.00	0.02	0.08	0.08	95.5	0.17	0.00
12.00	0.02	0.09	0.05	0.05	0.05	0.00	0.00	0.00	0.03	0.03	98.0	0.09	0.00
14.00	0.01	0.03	0.04	0.04					0.03	0.01	47.7	0.04	0.01

* Inhibition is expressed as the proportion of inhibited enzyme compared to baseline

Table 27

Erythrocyte Acetylcholinesterase Inhibition in Subjects
after Receiving One Standard Tablet While Fasting

Scheduled Interval	Subject 01	02	05	06	09	10	13	14	MEAN	SD	CV (%)	MAX	MIN
				Inhibition*									
0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	282.8	0.01	0.00
0.25	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	217.4	0.01	0.00
0.50	0.09	0.00	0.11	0.00	0.00	0.09	0.00	0.02	0.04	0.05	124.7	0.11	0.00
0.75	0.24	0.07	0.24	0.10	0.04	0.15	0.09	0.17	0.14	0.07	53.1	0.24	0.04
1.00	0.33		0.31	0.18	0.18	0.21	0.20	0.25	0.24	0.06	25.3	0.33	0.18
1.33	0.36	0.25	0.33	0.24	0.30	0.25	0.35	0.31	0.30	0.05	16.0	0.36	0.24
1.67	0.38	0.25	0.33	0.28	0.38	0.20	0.42	0.37	0.33	0.08	23.5	0.38	0.20
2.00	0.38	0.26	0.34	0.27	0.38	0.24	0.45	0.34	0.33	0.07	21.5	0.45	0.26
2.50	0.55	0.23	0.42	0.31	0.40	0.20	0.47	0.29	0.36	0.12	33.6	0.55	0.20
3.00	0.38	0.23	0.42	0.30	0.42	0.20	0.49	0.26	0.34	0.10	30.9	0.49	0.23
3.50	0.39	0.25	0.39	0.26	0.43	0.17	0.44	0.22	0.32	0.10	30.9	0.49	0.23
4.00	0.36	0.23	0.34	0.21	0.45	0.20	0.40	0.22	0.30	0.10	32.3	0.44	0.22
5.00	0.26	0.18	0.32	0.16	0.36	0.14	0.34	0.15	0.24	0.09	38.4	0.36	0.14
6.00	0.23	0.11	0.21	0.16	0.32	0.13	0.26	0.12	0.19	0.08	39.7	0.32	0.11
7.00	0.18	0.11	0.20	0.10	0.20	0.08	0.20	0.09	0.15	0.05	37.0	0.20	0.08
8.00	0.11	0.11	0.16	0.09	0.13	0.05	0.15	0.08	0.11	0.04	34.3	0.16	0.05
10.00	0.11	0.05	0.12	0.04	0.11	0.04	0.06	0.00	0.07	0.04	64.5	0.11	0.00
12.00	0.13	0.10	0.07	0.03	0.07	0.02	0.07		0.07	0.04	55.2	0.13	0.02
14.00	0.07		0.05	0.00	0.05	0.00	0.05	0.02	0.03	0.03	81.7	0.07	0.00
24.00	0.00	0.00	0.00	0.00	0.03	0.03	0.00	0.00	0.01	0.01	156.3	0.03	0.00

* Inhibition is expressed as the proportion of inhibited enzyme compared to baseline

Table 28

Erythrocyte Acetylcholinesterase Inhibition in Subjects
after Receiving One Sustained-Release Tablet while Fasting

Scheduled Interval (hours)	Subject 03	04	07	08	11	12	15	16	MEAN	SD	CV (%)	MAX	MIN
						Inhibition*							
0.00	0.00	0.02	0.02	0.02	0.00	0.00	0.01	0.00	0.01	0.01	113.3	0.02	0.00
0.25	0.04	0.00	0.01	0.01	0.00	0.00	0.01	0.03	0.01	0.01	114.1	0.04	0.00
0.50	0.20	0.10	0.04	0.00	0.04	0.04	0.08	0.06	0.07	0.06	84.0	0.20	0.00
0.75	0.29	0.21	0.11	0.03	0.07	0.14	0.10	0.19	0.14	0.08	57.4	0.29	0.03
1.00	0.36	0.33	0.14	0.15	0.09	0.20	0.26	0.19	0.22	0.10	44.3	0.36	0.09
1.33	0.42	0.47	0.18	0.30	0.15	0.23	0.43	0.33	0.31	0.12	38.7	0.43	0.15
1.67	0.42	0.57	0.16	0.34	0.19	0.27	0.50	0.38	0.35	0.14	40.6	0.50	0.16
2.00	0.38	0.61	0.21	0.38	0.25	0.41	0.49	0.44	0.40	0.13	31.8	0.49	0.21
2.50	0.36	0.52	0.22	0.55	0.32	0.49	0.41	0.49	0.41	0.11	26.7	0.55	0.22
3.00	0.37	0.49	0.24	0.52	0.30	0.44	0.37	0.39	0.39	0.09	23.8	0.52	0.24
3.50	0.35	0.45	0.28	0.45	0.26	0.41	0.32	0.37	0.36	0.07	19.8	0.45	0.26
4.00	0.33	0.44	0.31	0.42	0.25	0.39	0.24	0.30	0.33	0.07	22.1	0.44	0.24
5.00	0.27	0.38	0.30	0.35	0.24	0.30	0.21	0.26	0.29	0.06	19.3	0.45	0.21
6.00	0.21	0.36	0.21	0.26	0.17	0.21	MA	MA	0.24	0.07	27.7	0.32	0.10
7.00	0.11	0.24	0.16	0.21	0.08	0.17	MA	MA	0.16	0.06	36.6	0.29	0.08
8.00	0.11	0.18	0.08	0.15	0.08	0.12	0.18	0.16	0.13	0.04	29.2	0.27	0.08
10.00	0.06	0.14	0.10	0.09	0.04	0.07	0.13	0.13	0.09	0.04	38.6	0.17	0.04
12.00	0.01	0.09	0.06	0.05	0.01	0.05	0.18	0.13	0.07	0.06	80.2	0.18	0.01
14.00	0.02	0.08	0.03	0.01	0.00	0.03	0.13	0.14	0.05	0.05	98.8	0.14	0.00
24.00	0.03	0.07	0.00	0.00	0.00	0.00	0.07	0.06	0.03	0.03	116.7	0.07	0.00

MA = machine malfunction prevented assay from being performed

*Inhibition is expressed as the proportion of inhibited enzyme compared to baseline

Table 2a

Erythrocyte Acetylcholinesterase Inhibition in Subjects
after Receiving Standard Tablet with Food -- First Dose

Scheduled Interval (hours)	Subject 01	02	05	06	09	10	13	14	MEAN	SD	CV (%)	MAX	MIN
				Inhibition*									
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00
0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.01	0.01	282.8	0.04	0.00
0.50	0.05	0.01	0.00	0.00	0.00	0.00	0.05	0.21	0.04	0.07	177.0	0.21	0.00
0.75	0.10	0.10	0.00	0.02	0.00	0.01	0.14	0.27	0.08	0.10	119.0	0.27	0.00
1.00	0.18	0.15	0.00	0.05	0.00	0.09	0.18	0.32	0.12	0.11	90.0	0.32	0.00
1.33	0.21	0.17	0.03	0.10	0.07	0.14	0.20	0.33	0.16	0.10	61.5	0.33	0.03
1.67	0.21	0.21	0.07	0.15	0.10	0.18	0.25	0.32	0.19	0.08	43.0	0.32	0.07
2.00	0.26	0.23	0.14	0.21	0.18	0.22	0.27	0.29	0.23	0.05	22.5	0.29	0.14
2.50	0.29	0.23	0.25	0.25	0.23	0.24	0.27	0.29	0.26	0.02	9.4	0.29	0.23
3.00	0.30	0.22	0.22	0.24	0.24	0.25	0.28	0.21	0.24	0.03	12.4	0.30	0.21
3.50	0.28	0.20	0.20	0.23	0.28	0.26	0.29	0.19	0.24	0.04	16.7	0.29	0.19
4.00	0.30	0.24	0.21	0.23	0.27	0.23	0.29	0.14	0.24	0.05	21.9	0.30	0.14
5.00	0.27	0.24	0.20	0.17	0.27	0.22	0.27	0.10	0.22	0.06	28.3	0.27	0.10
6.00	0.17	0.16	0.15	0.14	0.24	0.19	0.23	0.09	0.17	0.05	27.6	0.24	0.09
7.00	0.12	0.12	0.13	0.16	0.18	0.14	0.22	0.10	0.15	0.04	26.9	0.22	0.10
8.00	0.12	0.09	0.10	0.13	0.14	0.11	0.20	0.09	0.12	0.04	20.1	0.20	0.08

*Inhibition is expressed as the proportion of inhibited enzyme compared to baseline

Table 30

Erythrocyte Acetylcholinesterase Inhibition in Subjects
after Receiving One Sustained-Release Tablet
with Food -- First Dose

Scheduled Interval (hours)	Subject 03	04	07	08	11	12	15	16	MEAN	SD	CV (%)	MAX	MIN
				Inhibition*									
0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	282.8	0.01	0.00
0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	282.8	0.00	0.00
0.50	0.01	0.00	0.05	0.00	0.00	0.02	0.02	0.00	0.01	0.02	131.9	0.05	0.00
0.75	0.10	0.10	0.03	0.03	0.00	0.16	0.12	0.00	0.07	0.06	90.0	0.16	0.00
1.00	0.14	MA	0.04	0.07	0.01	0.15	0.19	0.05	0.09	0.07	70.5	0.19	0.01
1.33	MA	MA	0.09	0.20	0.05	0.21	0.25	0.09	0.15	0.08	55.1	0.25	0.05
1.67	0.26	0.26	0.11	0.28	0.12	0.24	0.29	0.20	0.22	0.07	32.1	0.29	0.11
2.00	0.28	0.31	0.15	0.36	0.20	0.32	0.32	0.24	0.27	0.07	25.6	0.32	0.15
2.50	0.24	0.32	0.17	0.43	0.40	0.34	0.32	0.34	0.32	0.08	26.0	0.43	0.17
3.00	0.24	0.35	0.20	0.48	0.41	0.45	0.30	0.40	0.35	0.10	28.6	0.48	0.20
3.50	0.24	0.41	0.22	0.50	0.44	0.47	0.33	0.46	0.38	0.11	28.3	0.50	0.22
4.00	0.26	0.44	0.26	0.53	0.47	0.50	0.38	0.53	0.42	0.11	26.5	0.53	0.26
5.00	0.24	0.45	0.25	0.52	0.51	0.52	0.42	0.57	0.44	0.13	28.7	0.57	0.24
6.00	0.17	0.32	0.21	0.46	0.45	0.45	0.32	0.41	0.35	0.11	32.1	0.46	0.17
7.00	0.13	0.29	0.15	0.38	0.42	0.36	0.26	0.33	0.29	0.11	36.2	0.42	0.13
8.00	0.13	0.27	0.09	0.31	0.40	0.32	0.22	0.31	0.26	0.10	40.1	0.40	0.13
10.00	0.08	0.17	0.08	0.19	0.29	0.23	0.19	0.21	0.18	0.07	38.7	0.29	0.08
12.00	0.07	0.08	0.12	0.14	0.19	0.18	0.10	0.17	0.13	0.05	36.2	0.19	0.07

MA = machine malfunction prevented test from bein; performed

*Inhibition is expressed as the proportion of inhibited enzyme compared to baseline

Table 31

Erythrocyte Acetylcholinesterase Inhibition in Subjects after Receiving One Standard Tablet on Day 2 of Multiple-Dose Portion

Scheduled Interval (hours)	Subject 01	02	05	06	09	10	13	14	MEAN	SD	CV (%)	MAX	MIN
				Inhibition*									
0.00	0.24	0.08	0.19	0.06	0.17	0.26	0.18	0.12	0.16	0.07	44.0	0.26	0.06
0.25	0.24	0.08	0.22	0.12	0.15	0.22	0.15	0.07	0.16	0.07	41.9	0.24	0.07
0.50	0.31	0.12	0.25	0.10	0.23	0.25	0.15	0.13	0.19	0.08	39.5	0.31	0.10
0.75	0.33	0.13	0.23	0.11	0.30	0.26	0.19	0.22	0.22	0.08	35.3	0.33	0.11
1.00	0.31	0.16	0.23	0.12	0.38	0.31	0.21	0.26	0.25	0.08	34.1	0.38	0.12
1.33	0.32	0.16	0.23	0.16	0.36	0.40	0.25	0.28	0.27	0.09	31.8	0.40	0.16
1.67	0.31	0.17	0.23	0.20	MA	MA	0.27	0.28	0.24	0.05	20.8	0.31	0.17
2.00	0.28	0.18	0.24	0.22	0.38	0.37	0.37	0.37	0.30	0.08	27.0	0.38	0.18
2.50	0.26	0.18	0.32	0.36	0.35	0.36	0.38	0.38	0.33	0.07	22.0	0.38	0.18
3.00	0.29	0.24	0.30	0.35	0.39	0.34	0.40	0.37	0.34	0.06	16.8	0.40	0.24
3.50	0.30	0.23	0.33	0.36	0.36	0.34	0.38	0.34	0.33	0.05	13.9	0.38	0.23
4.00	0.27	0.25	0.36	0.35	0.31	0.29	0.37	0.31	0.31	0.04	13.8	0.37	0.25
5.00	0.27	0.20	0.34	0.34	0.25	0.29	0.35	0.26	0.29	0.05	18.1	0.35	0.20
6.00	0.22	0.17	0.30	0.29	0.24	0.30	0.29	0.19	0.25	0.05	21.1	0.30	0.17
7.00	0.22	0.13	0.27	0.23	0.18	0.23	0.29	0.17	0.21	0.05	24.5	0.29	0.13
8.00	0.17	0.10	0.22	0.23	0.18	0.19	0.26	0.17	0.19	0.05	26.0	0.26	0.10
24.00	0.22	0.04	0.25	0.14	0.13	0.24	0.26	0.22	0.19	0.08	42.0	0.26	0.04

*Inhibition is expressed as the proportion of inhibited enzyme compared to baseline

MA = machine malfunction prevented test from being performed

Table 32

Erythrocyte Acetylcholinesterase Inhibition in Subjects
after Receiving One Sustained-Release Tablet on Day 2
of the Multiple-Dose Portion

Scheduled Interval (hours)	Subject 03	04	07	08	11	12	15	16	MEAN	SD	CV (%)	MAX	MIN
			Inhibition*										
0.00	0.18	0.21	0.28	0.24	0.17	0.34	0.24	0.25	0.24	0.05	23.0	0.34	0.17
0.25	0.32	MA	0.27	0.22	0.15	0.34	0.21	0.25	0.25	0.07	26.3	0.32	0.00
0.50	MA	MA	0.28	0.24	0.17	0.36	0.26	0.27	0.26	0.06	23.0	0.28	0.02
0.75	MA	MA	0.33	0.26	0.19	0.38	0.28	0.25	0.28	0.06	22.7	0.33	0.16
1.00	0.41	MA	0.31	0.30	0.25	0.40	0.31	0.28	0.32	0.06	18.0	0.41	0.15
1.33	0.43	0.33	0.35	0.33	0.30	0.40	0.31	0.27	0.34	0.05	15.4	0.43	0.21
1.67	0.42	0.34	0.38	0.33	0.36	0.42	0.37	0.32	0.37	0.04	10.1	0.42	0.24
2.00	0.44	0.36	0.38	0.33	0.40	0.47	0.34	0.32	0.38	0.05	14.3	0.44	0.32
2.50	0.47	0.35	0.40	0.30	0.47	0.47	0.39	0.30	0.39	0.07	18.2	0.47	0.30
3.00	0.47	0.36	0.42	0.30	0.46	0.51	0.38	0.30	0.40	0.08	19.5	0.47	0.30
3.50	0.48	0.39	0.44	0.29	0.49	0.54	0.37	0.27	0.41	0.10	23.6	0.49	0.27
4.00	0.49	0.37	MA	0.23	0.52	0.56	0.35	0.24	0.39	0.13	33.7	0.52	0.23
5.00	0.51	0.41	0.48	0.23	0.52	0.53	0.36	0.22	0.41	0.13	31.6	0.52	0.22
6.00	0.42	0.36	0.41	0.17	0.49	0.43	0.30	0.19	0.35	0.12	33.5	0.49	0.19
7.00	0.40	0.32	0.32	0.12	0.43	0.36	0.25	0.20	0.30	0.10	35.0	0.43	0.12
8.00	0.38	0.36	0.27	0.16	0.40	0.28	0.17	0.16	0.27	0.10	37.1	0.40	0.16
10.00	0.33	0.23	0.29	0.10	0.37	0.31	0.16	0.14	0.24	0.10	40.9	0.37	0.10
12.00	0.27	0.20	0.20	0.09	0.27	0.20	0.12	0.16	0.19	0.07	34.8	0.27	0.12
24.00	0.38	0.21	0.08	0.22	0.38	0.24	0.29	0.20	0.25	0.10	40.2	0.38	0.08

*Inhibition is expressed as the proportion of inhibited enzyme compared to baseline

MA = machine malfunction prevented test from being performed

Table 33

Bioavailability of Pyridostigmine Formulations
Determined From the Area Under the Red Blood Cell
Acetylcholinesterase Inhibition Time Curve

STANDARD TABLET

	SUBJECT 1	2	5	6	9	10	13	14	MEAN	SD	CV (%)	MAX	MIN
AUC-IV	1.97	1.89	2.69	2.21	2.69	2.19	2.59	2.00	2.28	0.33	14.57	2.69	1.89
AUC-FASTING	2.92	1.64	2.82	1.63	2.89	1.33	2.87	1.54	2.21	0.72	32.79	2.92	1.33
AUC-FIRST DOSE FED	1.62	1.36	1.11	1.26	1.48	1.38	1.83	1.35	1.42	0.22	15.56	1.83	1.11
AUC-STEADY STATE FED	2.09	1.38	2.28	2.11	2.25	2.37	2.45	2.03	2.12	0.33	15.65	2.45	1.38
DOSE IV ug pyridostigmine base	3984	4272	4008	3936	4272	4248	4368	4440					
DOSE PO ug pyridostigmine base	20889	20889	20889	20889	20889	20889	20889	20889					
BIOAVAILABILITY-FASTING	0.28	0.18	0.20	0.14	0.22	0.12	0.23	0.16	0.19	0.05	27.21	0.28	0.12
BIOAVAILABILITY-FED-SS	0.20	0.15	0.16	0.18	0.17	0.22	0.20	0.22	0.19	0.03	13.67	0.22	0.15
FED BIOAVAIL/FASTING BIOAVAIL	0.72	0.84	0.81	1.29	0.78	1.78	0.85	1.32	1.05	0.38	35.91	1.78	0.72
ACCUMULATION-FED-SS/1ST DOSE	1.29	1.01	2.05	1.67	1.52	1.72	1.34	1.50	1.51	0.31	20.72	2.05	1.01

SUSTAINED-RELEASE TABLET

	SUBJECT 3	4	7	8	11	12	15	16	MEAN	SD	CV (%)	MAX	MIN
AUC-IV	2.43	2.44	2.41	2.53	2.84	2.18	1.78	2.22	2.35	0.31	13.06	2.84	1.78
AUC-FASTING	2.40	3.63	2.03	2.83	1.61	2.52	2.78	2.77	2.57	0.60	23.33	3.63	1.61
AUC-FIRST DOSE FED	1.88	3.08	1.68	3.83	3.87	3.82	2.97	3.58	3.09	0.88	28.42	3.87	1.68
AUC-STEADY STATE FED	4.77	3.77	4.13	2.32	4.75	4.63	3.10	2.53	3.75	0.99	26.51	4.77	2.32
DOSE IV ug pyridostigmine base	4008	4176	4488	4224	4440	4440	4512	4368					
DOSE PO ug pyridostigmine base	64056	64056	64056	64056	64056	64056	64056	64056					
BIOAVAILABILITY-FASTING	0.06	0.10	0.06	0.07	0.04	0.08	0.11	0.09	0.08	0.02	29.68	0.11	0.04
BIOAVAILABILITY-FED-SS	0.12	0.10	0.12	0.06	0.12	0.15	0.12	0.08	0.11	0.03	25.63	0.15	0.06
FED BIOAVAIL/FASTING BIOAVAIL	1.99	1.04	2.03	0.82	2.95	1.84	1.12	0.91	1.59	0.74	46.72	2.95	0.82
ACCUMULATION-FED-SS/1ST DOSE	2.54	1.22	2.46	0.61	1.23	1.21	1.04	0.71	1.38	0.73	53.11	2.54	0.61

AUC are fraction inhibited x hr x ml^{-1}

AUC-IV and AUC-Fasting are to last measured concentration. AUC-First Dose and AUC-Steady State Fed are for 8 hour interval in the case of the standard preparation and 12 hours for the sustained release preparation.

Table 34

Intravenous Infusion in Subjects Receiving Standard Tablet:
Time When Erythrocyte Acetylcholinesterase Inhibition
Exceeded 0.20 AND 0.40

SUBJECT	EARLIEST TIME ≥ 0.20 INHIBITION	LATEST TIME ≥ 0.20 INHIBITION	DURATION ≥ .20	EARLIEST TIME ≥ 0.40 INHIBITION	LATEST TIME ≥ 0.40 INHIBITION	DURATION ≥ .40	DURATION ≥ 0.20 & ≤ 0.40
01	1.00	6.50	5.50	N/A	N/A	0.00	5.50
02	1.50	6.25	4.75	N/A	N/A	0.00	4.75
05	1.00	7.00	6.00	N/A	N/A	0.00	6.00
06	1.50	6.50	5.00	N/A	N/A	0.00	5.00
09	1.00	7.00	6.00	N/A	N/A	0.00	6.00
10	1.03	6.50	5.47	N/A	N/A	0.00	5.47
13	1.00	7.00	6.00	N/A	N/A	0.00	6.00
14	1.50	6.50	5.00	N/A	N/A	0.00	5.00
MEAN			5.47			0.00	5.47
SD			0.51			0.00	0.51
CV (%)			9.29			0.00	9.29
MAX			6.00			0.00	6.00
MIN			4.75			0.00	4.75

Table 35

Intravenous Infusion in Subjects Receiving Sustained-Release Tablet
Time When Erythrocyte Acetylcholinesterase Inhibition
Exceeded 0.20 AND 0.40

SUBJECT	EARLIEST TIME ≥ 0.20 INHIBITION	LATEST TIME ≥ 0.20 INHIBITION	DURATION ≥ .20	EARLIEST TIME ≥ 0.40 INHIBITION	LATEST TIME ≥ 0.40 INHIBITION	DURATION ≥ .40	DURATION ≥ 0.20 & ≤ 0.40
03	1.50	7.00	5.50	N/A	N/A	0.00	5.50
04	1.50	6.50	5.00	N/A	N/A	0.00	5.00
07	1.50	6.50	5.00	N/A	N/A	0.00	5.00
08	3.00	7.00	4.00	N/A	N/A	0.00	4.00
11	1.00	7.00	6.00	3.00	5.00	2.00	4.00
12	1.00	6.50	5.50	N/A	N/A	0.00	5.50
15	1.50	6.33	4.83	N/A	N/A	0.00	4.83
16	1.50	6.50	5.00	N/A	N/A	0.00	5.00
MEAN			5.10			0.25	4.85
SD			0.59			0.71	0.58
CV (%)			11.57			282.84	11.96
MAX			6.00			2.00	5.50
MIN			4.00			0.00	4.00

Table 36

Standard Tablet Given Fasting:
Time When Erythrocyte Acetylcholinesterase Inhibition
Exceeded 0.20 AND 0.40

SUBJECT	EARLIEST TIME ≥ 0.20 INHIBITION	LATEST TIME ≥ 0.20 INHIBITION	DURATION ≥ .20	EARLIEST TIME ≥ 0.40 INHIBITION	LATEST TIME ≥ 0.40 INHIBITION	DURATION ≥ .40	DURATION ≥ 0.20 & ≤ 0.40
01	0.75	6.00	5.25	2.50	2.50	0.00	5.25
02	1.33	4.00	2.67	N/A	N/A	0.00	2.67
05	0.75	7.00	6.25	2.50	0.50	0.50	5.75
06	1.33	4.00	2.67	N/A	3.00	0.00	2.67
09	1.33	7.00	5.67	2.50	N/A	1.50	4.17
10	1.00	4.00	3.00	N/A	N/A	0.00	3.00
13	1.00	7.00	6.00	1.67	4.00	2.33	3.67
14	1.00	4.00	3.00	N/A	N/A	0.00	3.00
MEAN			4.31			0.54	3.77
SD			1.61			0.89	1.19
CV (%)			37.34			165.03	31.50
MAX			6.25			2.33	5.75
MIN			2.67			0.00	2.67

Table 37

Sustained-Release Tablet Given Fasting:
Time When Erythrocyte Acetylcholinesterase Inhibition
Exceeded 0.20 AND 0.40

SUBJECT	EARLIEST TIME ≥ 0.20 INHIBITION	LATEST TIME ≥ 0.20 INHIBITION	DURATION ≥ .20	EARLIEST TIME ≥ 0.40 INHIBITION	LATEST TIME ≥ 0.40 INHIBITION	DURATION ≥ .40	DURATION ≥ 0.20 & ≤ 0.40
03	0.50	6.02	5.52	1.33	1.67	0.34	5.18
04	0.75	7.03	6.28	1.33	4.00	2.67	3.61
07	2.00	6.00	4.00	N/A	N/A	0.00	4.00
08	1.33	7.00	5.67	2.50	4.00	1.50	4.17
11	2.00	5.00	3.00	N/A	N/A	0.00	3.00
12	1.00	6.00	5.00	2.00	3.50	1.50	3.50
15	1.00	5.00	4.00	1.33	2.50	1.17	2.83
16	1.35	5.00	3.65	2.00	2.52	0.52	3.13
MEAN			4.64			0.96	3.68
SD			1.14			0.92	0.77
CV (%)			24.63			95.97	20.85
MAX			6.28			2.67	5.18
MIN			3.00			0.00	2.83

Table 38

Standard Tablet Given with Food -- First Dose:
Time When Erythrocyte Acetylcholinesterase Inhibition
Exceeded 0.20 AND 0.40

SUBJECT	EARLIEST TIME ≥ 0.20 INHIBITION	LATEST TIME ≥ 0.20 INHIBITION	DURATION ≥ .20	EARLIEST TIME ≥ 0.40 INHIBITION	LATEST TIME ≥ 0.40 INHIBITION	DURATION ≥ .40	DURATION ≥ 0.20 & ≤ 0.40
01	1.33	5.00	3.67	N/A	N/A	0.00	3.67
02	1.67	5.00	3.33	N/A	N/A	0.00	3.33
05	2.50	5.00	2.50	N/A	N/A	0.00	2.50
06	2.00	4.00	2.00	N/A	N/A	0.00	2.00
09	2.50	7.00	4.50	N/A	N/A	0.00	4.50
10	2.00	5.00	3.00	N/A	N/A	0.00	3.00
13	1.33	7.00	5.67	N/A	N/A	0.00	5.67
14	0.50	3.00	2.50	N/A	N/A	0.00	2.50
MEAN			3.40			0.00	3.40
SD			1.21			0.00	1.21
CV (%)			35.51			0.00	35.51
MAX			5.67			0.00	5.67
MIN			2.00			0.00	2.00

Table 3a

Sustained-Release Tablet Given with Food -- First Dose:
Time When Erythrocyte Acetylcholinesterase Inhibition
Exceeded 0.20 AND 0.40

SUBJECT	EARLIEST TIME ≥ 0.20 INHIBITION	LATEST TIME ≥ 0.20 INHIBITION	DURATION ≥ .20	EARLIEST TIME ≥ 0.40 INHIBITION	LATEST TIME ≥ 0.40 INHIBITION	DURATION ≥ .40	DURATION ≥ 0.20 & ≤ 0.40
03	1.67	5.00	3.33	N/A	N/A	0.00	3.33
04	1.67	8.00	6.33	3.50	5.00	1.50	4.83
07	3.00	6.00	3.00	N/A	N/A	0.00	3.00
08	1.33	8.00	6.67	2.50	6.00	3.50	3.17
11	2.00	10.00	8.00	2.58	8.00	5.42	2.58
12	1.33	10.00	8.67	3.00	6.00	3.00	5.67
15	1.33	8.00	6.67	5.00	5.00	0.00	6.67
16	1.67	10.00	8.33	6.00	6.00	3.00	5.33
MEAN			6.27			2.35	4.46
SD			1.97			1.95	1.54
CV (%)			31.45			83.25	34.55
MAX			8.67			5.42	6.67
MIN			2.50			0.00	2.58

Table 40

Standard Tablet Given on Day 2 of Multiple-Dose Portion:
Time When Erythrocyte Acetylcholinesterase Inhibition
Exceeded 0.20 AND 0.40

SUBJECT	EARLIEST TIME ≥ 0.20 INHIBITION	LATEST TIME ≥ 0.20 INHIBITION	DURATION ≥ .20	EARLIEST TIME ≥ 0.40 INHIBITION	LATEST TIME ≥ 0.40 INHIBITION	DURATION ≥ .40	DURATION ≥ 0.20 & ≤ 0.40
01	0.00	7.00	7.00	N/A	N/A	0.00	7.00
02	3.00	5.00	2.00	N/A	N/A	0.00	2.00
05	0.25	8.00	7.75	N/A	N/A	0.00	7.75
06	1.67	8.00	6.33	N/A	N/A	0.00	6.33
09	0.50	6.00	5.50	N/A	N/A	0.00	5.50
10	0.00	7.00	7.00	1.33	1.33	0.00	7.00
13	0.75	7.00	6.25	3.00	3.00	0.00	6.25
14	0.75	5.00	4.25	N/A	N/A	0.00	4.25
MEAN			5.58			0.00	5.58
SD			1.19			0.00	1.91
CV (%)			34.20			0.00	34.20
MAX			7.75			0.00	7.75
MIN			2.00			0.00	2.00

Table 41

Sustained-Release Tablet Given on Day 2 of Multiple-Dose Portion:
Time When Erythrocyte Acetylcholinesterase Inhibition
Exceeded 0.20 AND 0.40

SUBJECT	EARLIEST TIME ≥ 0.20 INHIBITION	LATEST TIME ≥ 0.20 INHIBITION	DURATION ≥ .20	EARLIEST TIME ≥ 0.40 INHIBITION	LATEST TIME ≥ 0.40 INHIBITION	DURATION ≥ .40	DURATION ≥ 0.20 & ≤ 0.40
03	0.25	12.00	11.75	1.00	7.00	5.00	6.75
04	0.00	10.00	10.00	5.00	5.00	0.00	10.00
07	0.00	12.00	12.00	2.50	6.00	3.50	8.50
08	0.00	5.00	5.00	N/A	N/A	0.00	5.00
11	1.00	12.00	11.00	2.00	8.00	6.00	7.00
12	0.00	12.00	12.00	1.00	6.00	5.00	7.00
15	0.00	7.00	7.00	N/A	N/A	0.00	7.00
16	0.00	7.00	7.00	N/A	N/A	0.00	7.00
MEAN			9.14			2.07	7.07
SD			2.77			2.66	1.77
CV (%)			30.25			128.23	25.05
MAX			12.00			6.00	10.00
MIN			5.00			0.00	5.00

Figure 1

Red Blood Cell Acetylcholinesterase Inhibition
with IV Infusion of Pyridostigmine

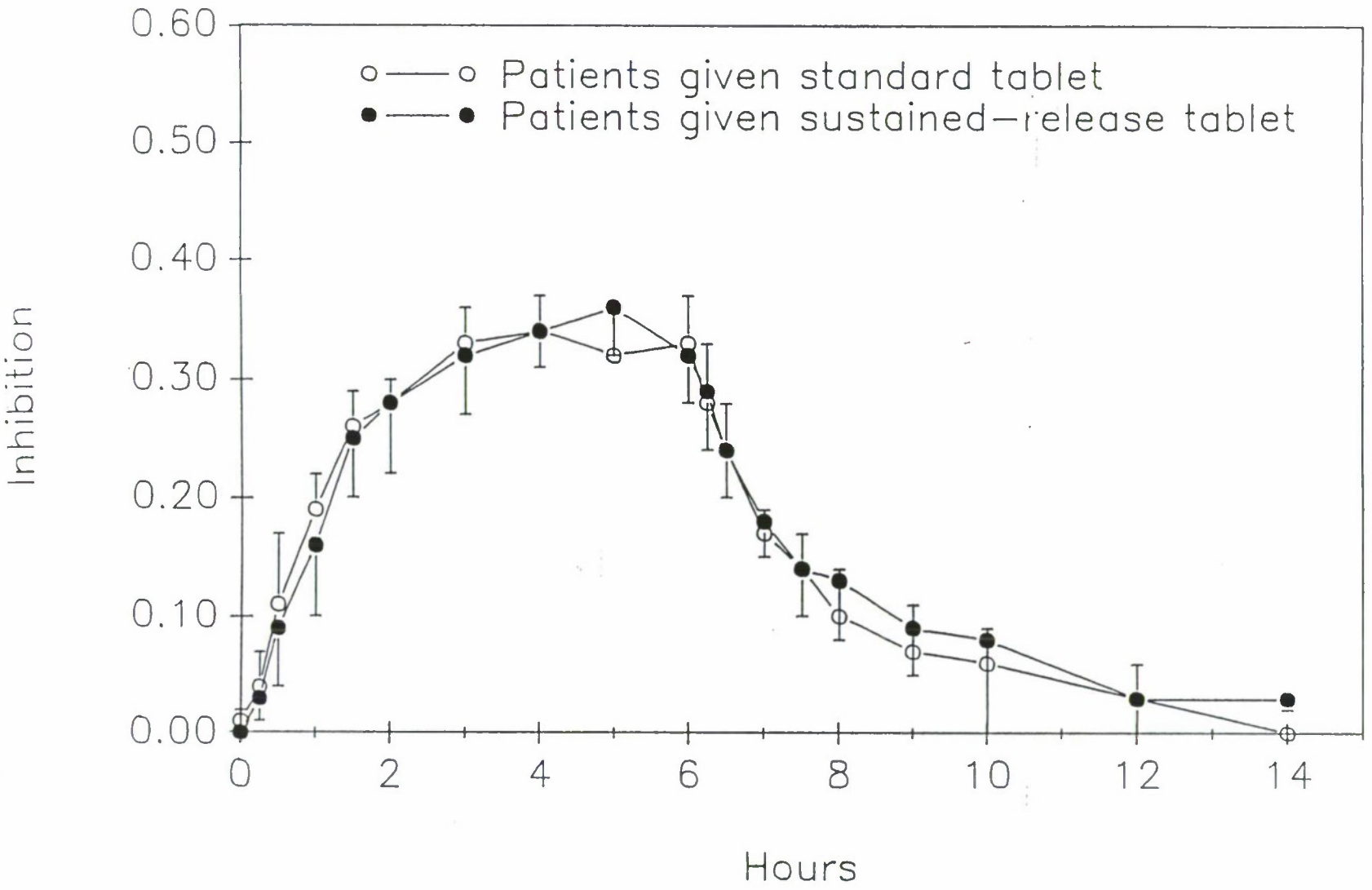


Figure 2

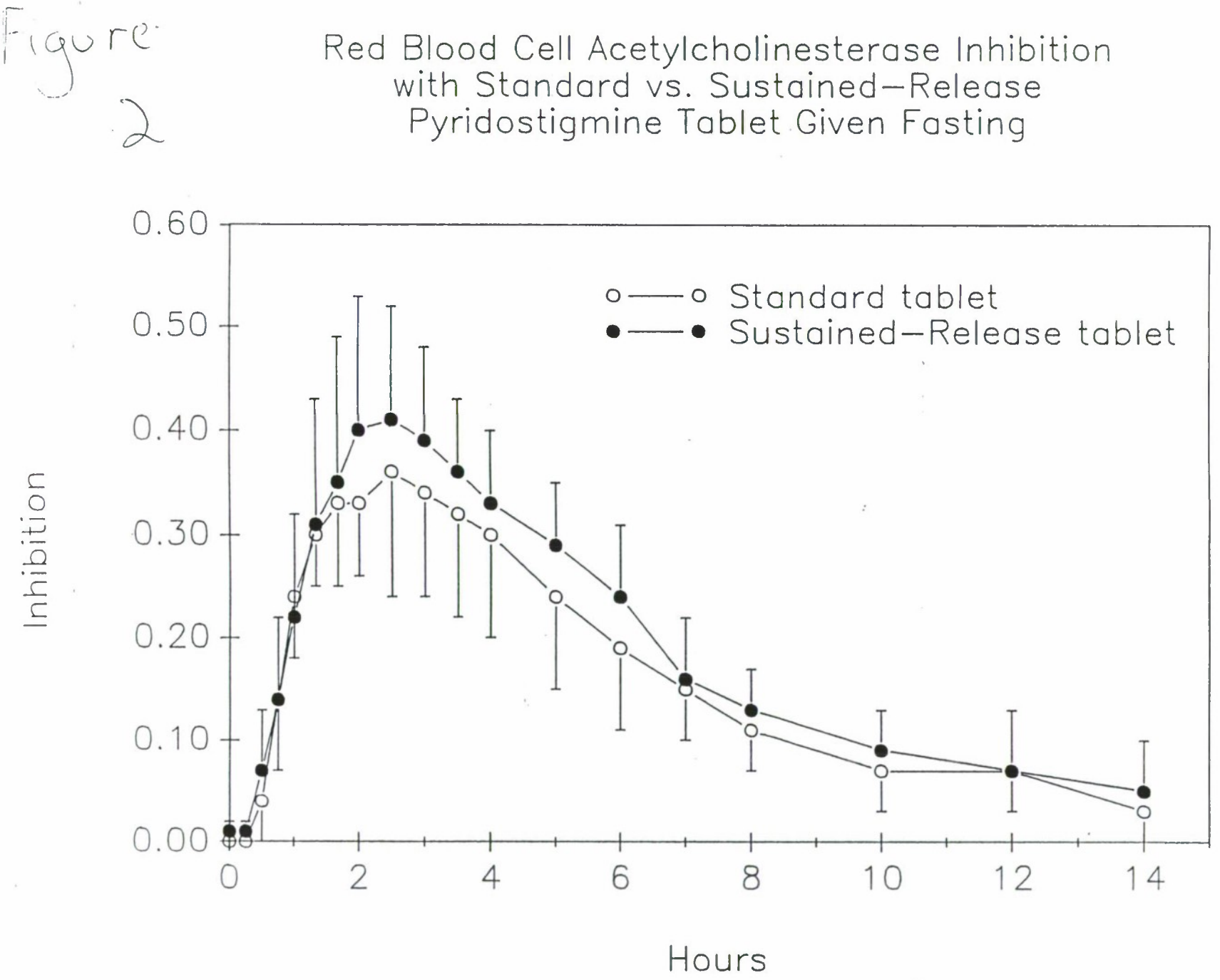

Red Blood Cell Acetylcholinesterase Inhibition
with Standard vs. Sustained–Release
Pyridostigmine Tablet Given Fasting
Standard tablet
Sustained–Release tablet
Inhibition
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
2
4
6
8
10
12
14
Hours

Figure 3

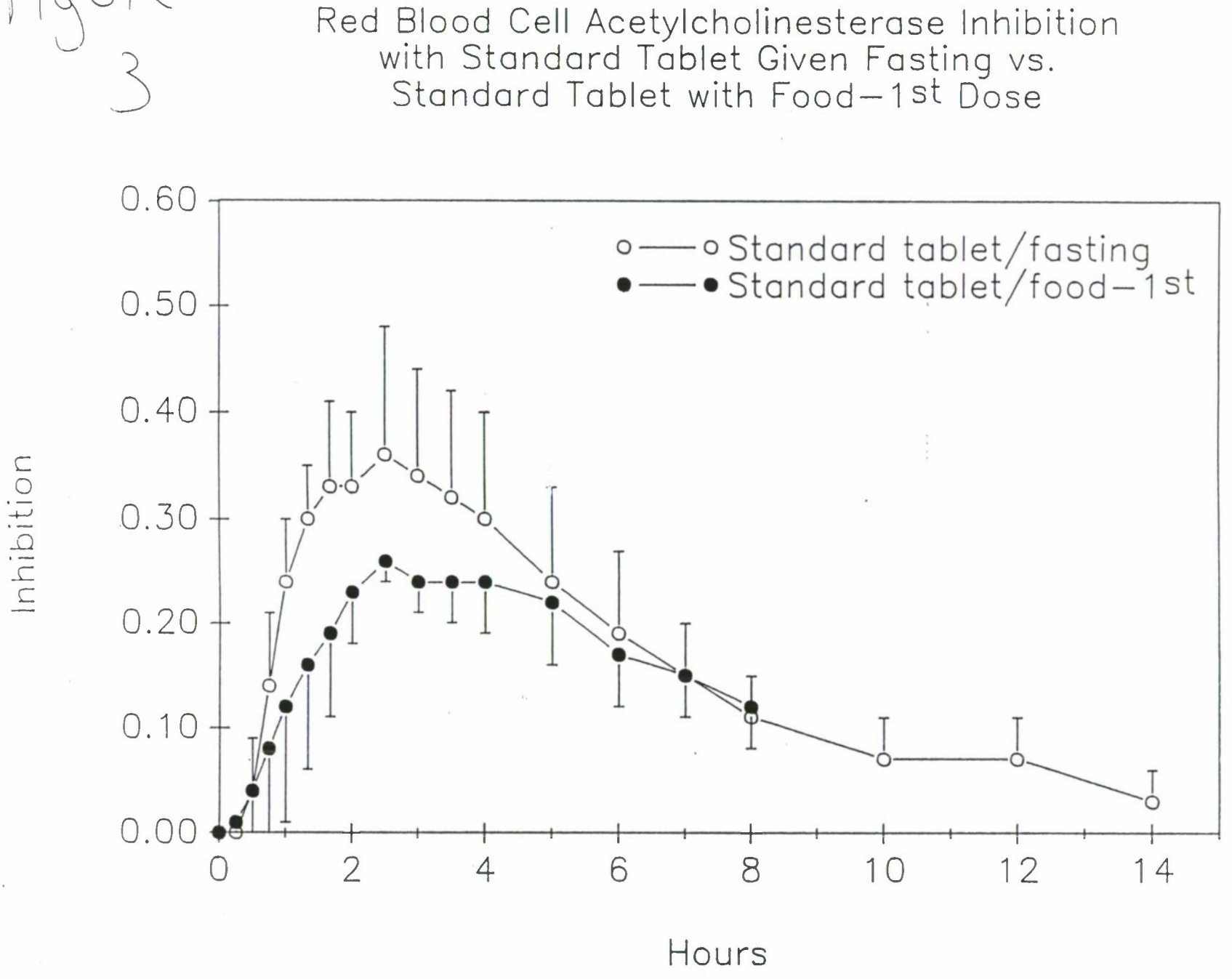

Red Blood Cell Acetylcholinesterase Inhibition
with Standard Tablet Given Fasting vs.
Standard Tablet with Food–1st Dose
Standard tablet/fasting
Standard tablet/food–1st
Inhibition
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0
2
4
6
8
10
12
14
Hours

Figure 4

Red Blood Cell Acetylcholinesterase Inhibition with Sustained–Release Tablet Given Fasting vs. Sustained–Release Tablet Given with Food–1st Dose

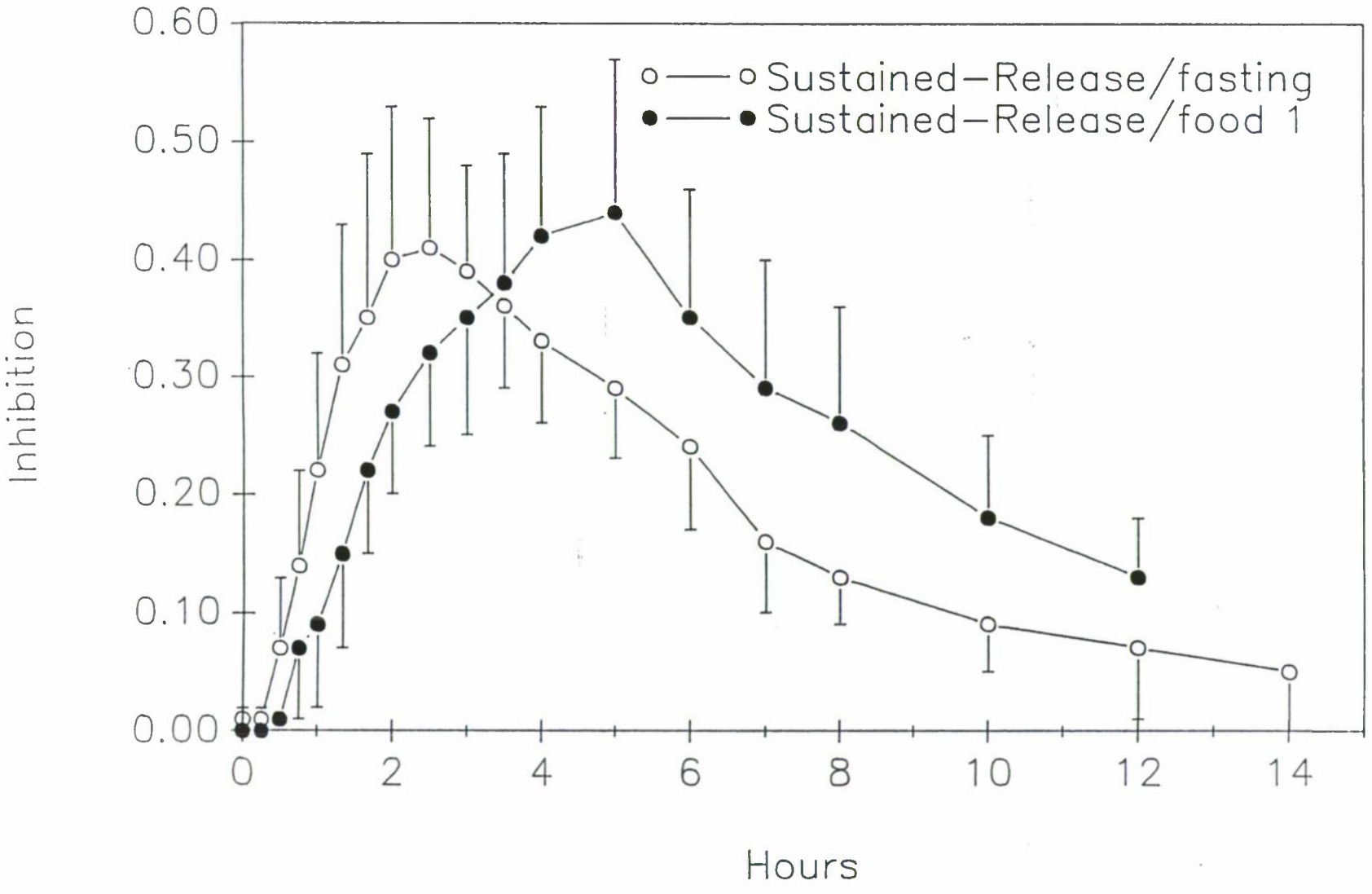


Figure 5

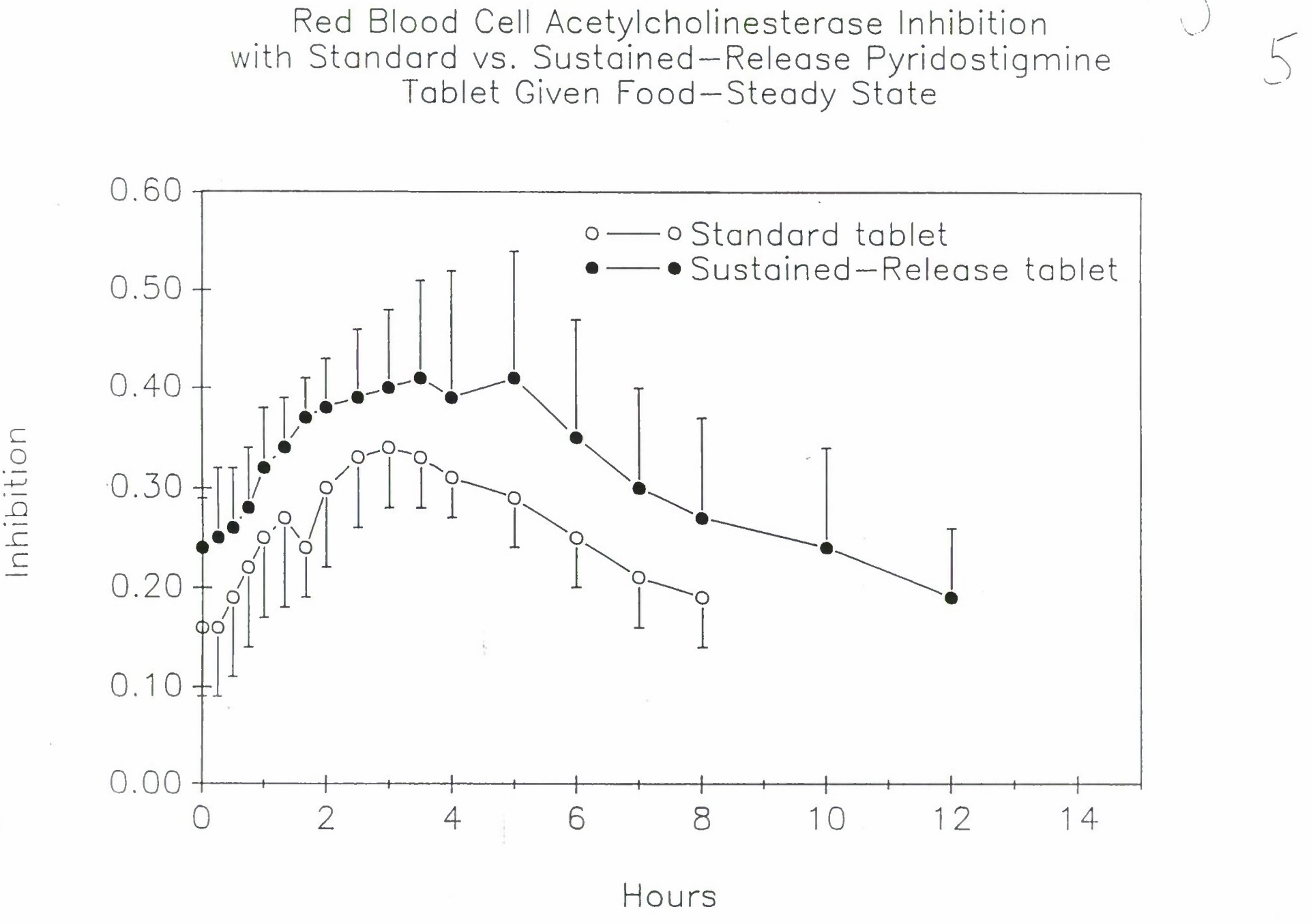

Red Blood Cell Acetylcholinesterase Inhibition
with Standard vs. Sustained–Release Pyridostigmine
Tablet Given Food–Steady State
Standard tablet
Sustained–Release tablet
Inhibition
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0
2
4
6
8
10
12
14
Hours

Figure 6

Red Blood Cell Acetylcholinesterase Inhibition with Standard vs. Sustained-Release Pyridostigmine Tablet Given with Food-1st Dose

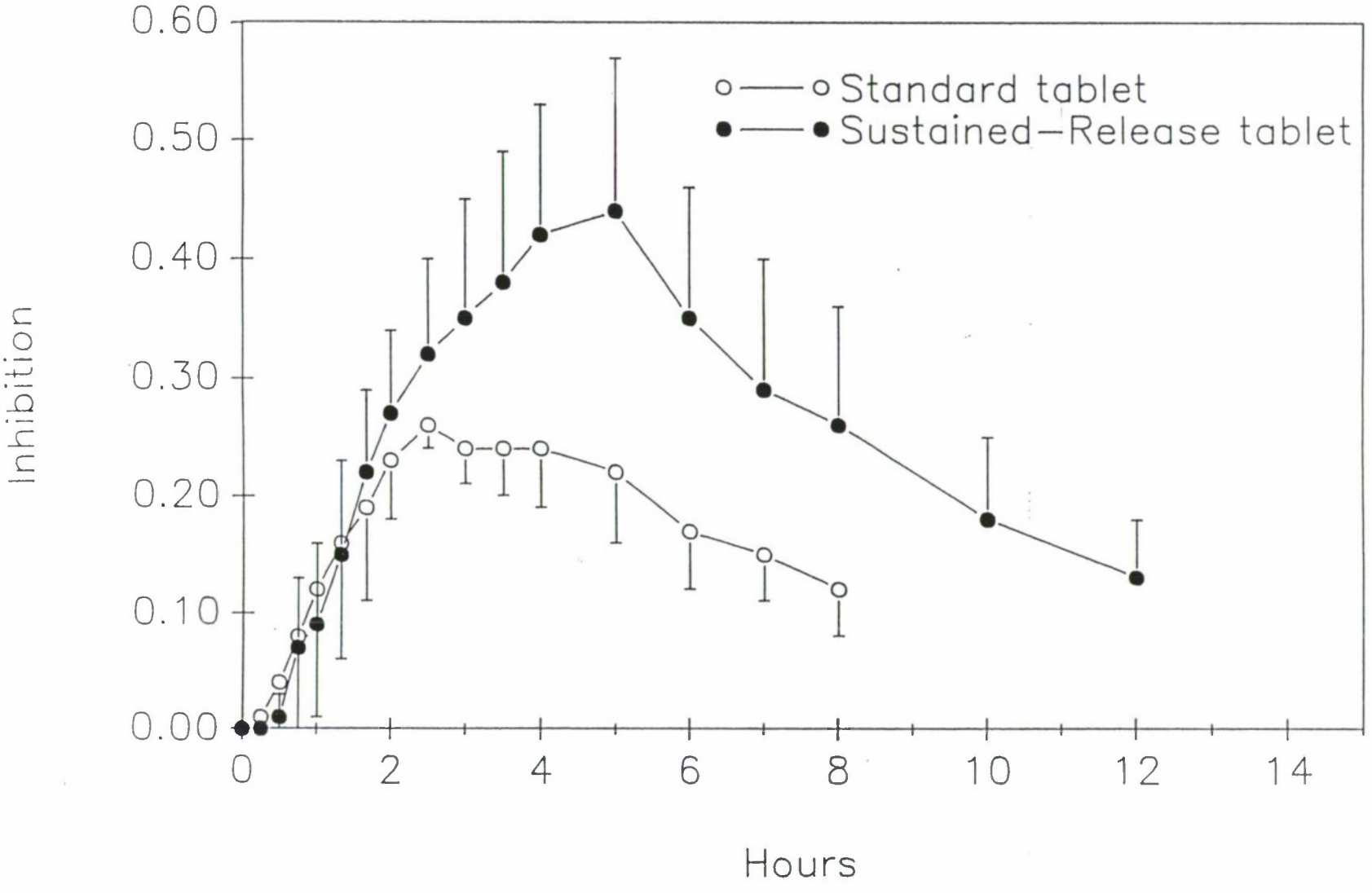


Figure 7

Red Blood Cell Acetylcholinesterase Inhibition
with Standard Tablet Given Fasting vs.
Standard Tablet Given with Food at Steady State

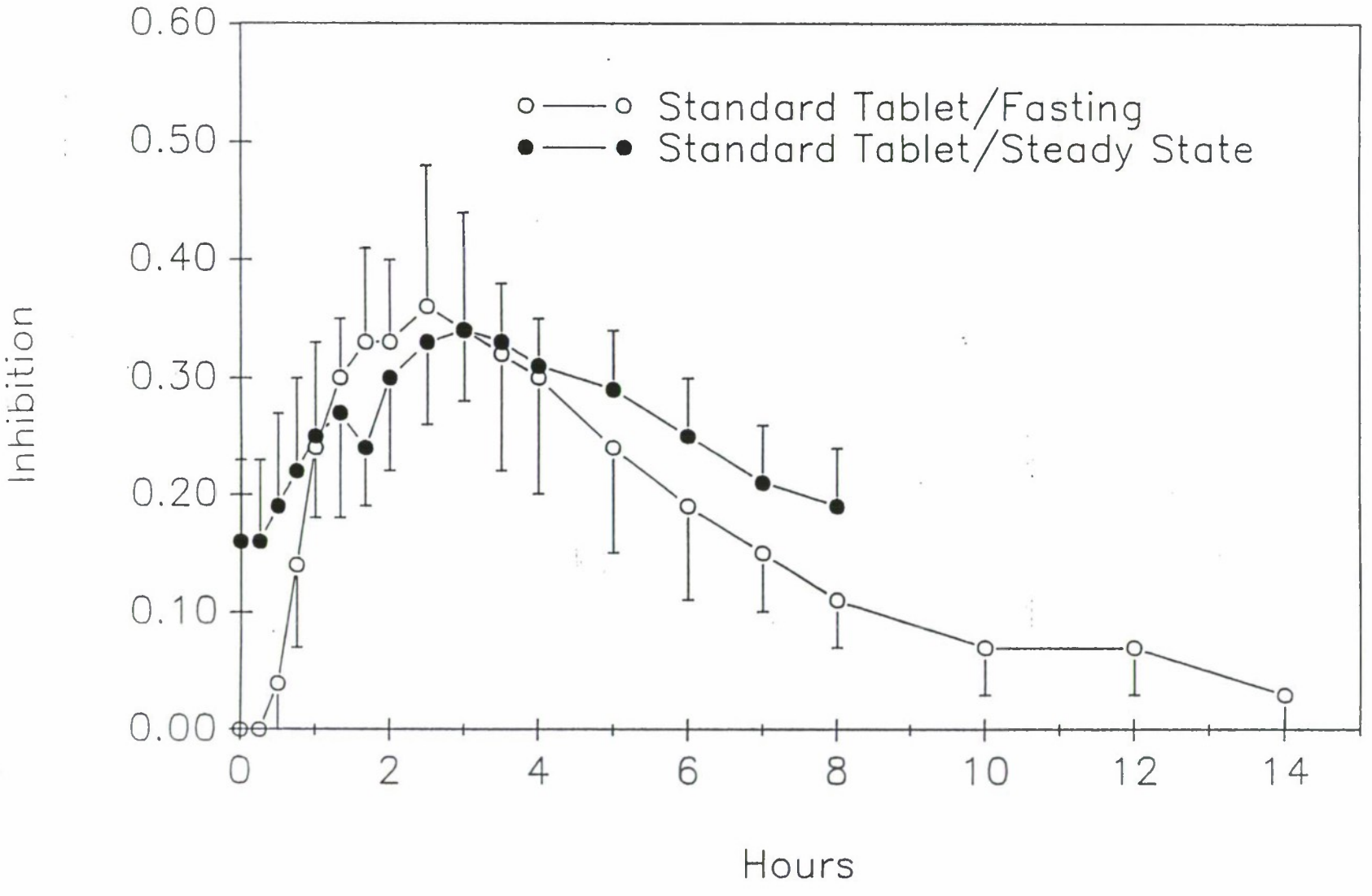
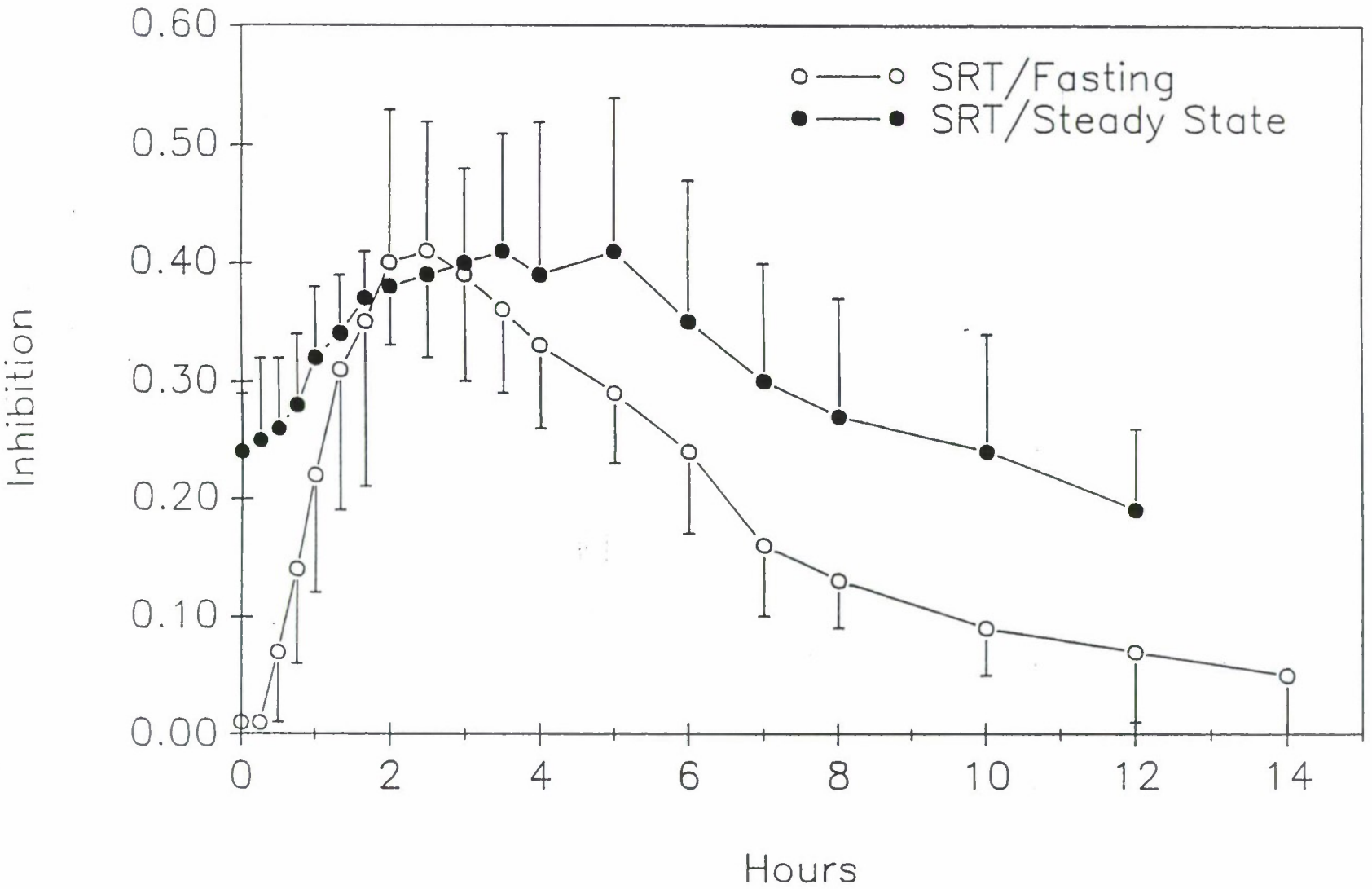


Figure 8

Red Blood Cell Acetylcholinesterase Inhibition
With Sustained–Release Tablet (SRT) Given Fasting vs.
Sustained–Release Tablet Given with Food at Steady State



CLINICAL INVESTIGATION CONSENT FORM

The Johns Hopkins Medical Institutions
(The Johns Hopkins Hospital
The Francis Scott Key Medical Center, etc.)

Patient I.D. Plate

Title of Research Project: Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Intravenous Pyridostigmine and Oral Doses of Standard and Sustained-Release Pyridostigmine in Healthy Men and the Influence of Food on Oral Pyridostigmine Pharmacokinetics

Explanation of Research Project to Subject:

You are invited to participate in a study of new forms of a drug called pyridostigmine. Pyridostigmine is a medicine that has been prescribed for patients for over 20 years. This drug is used indefinitely in several daily doses for treating patients with a disease called myasthenia gravis. The drug, based on studies in animals, may also be effective pre-treatment for accidental poisoning with certain insecticides which work in ways similar to nerve gases. This use of the drug is considered investigational by the Food and Drug Administration. Pyridostigmine is considered an experimental drug in this project because of the new use for which it is intended and because it is now being administered in a new formulation. The dose of the drug you will receive is smaller than the dose usually used in treating patients. The U.S. Army is funding this study to obtain information regarding the potential utility of pyridostigmine in the prevention of nerve gas toxicity. One of the new forms of pyridostigmine is hoped to be sustained-release; that is, absorbed into the body for a longer period of time than the standard form. This study is designed to compare the absorption of sustained-released pyridostigmine tablets with standard pyridostigmine tablets when given to healthy men, and to compare the effect of the oral tablets with intravenous pyridostigmine given for 6 hours.

If you agree to join this study you will be hospitalized for 6 full days. You will receive a slow intravenous infusion of pyridostigmine for 6 hours on the second day of hospitalization, and a single dose of either standard or sustained-release pyridostigmine on the third day. On the fifth day you will start taking the standard or sustained-release tablet 2 or 3 times a day for two days. A series of blood samples will be drawn from a "heparin lock," a small hollow plastic tube like an intravenous catheter that stays in a vein in your arm. This allows us to take repeated blood samples without sticking a new needle into your vein each time. If the heparin lock fails, however, we may obtain the necessary blood specimens by using a needle and syringe each time. The total blood taken for this study will be slightly more than that routinely donated at a blood bank. Because the blood taken in the study is spread over 6 days, you should not encounter any problems. Nevertheless, you should not donate blood or enter other studies for at least eight weeks after the conclusion of this study.

We believe that the risks of participation in this study are small. Pyridostigmine is considered an experimental drug in this project because of the new use for which it is intended. The dose of the drug you will receive is much smaller than the dose usually used in treating patients. In previous studies similar to this one, the only side effects noted were temporary fatigue, muscle twitching, and gastric distress. Patients who take more than 3 times as much of the drug each day than you will in this study sometimes

THIS CONSENT FORM CONTINUES ON THE REVERSE SIDE

develop nausea, vomiting, diarrhea, abdominal cramps, and increased body secretions. Because the dose you will receive is smaller than what doctors use in patients, it is not likely that you will develop these symptoms. Treatment is available if any such symptoms occur and become severe. There is little risk and only mild discomfort associated with the heparin lock or repeated blood sampling by using a needle and syringe.

Previously, a potential toxicity in rats was discovered which may be related to the drug. The rats were given pyridostigmine in a higher dosage than the one planned for this study. A change in muscle tissues in the area where the nerves and muscles meet was seen using an electron microscope. The significance of this finding is unknown, but it was reversible and it is generally accepted in the medical field that pyridostigmine is safe for man at the doses to be used in this study. Further testing was done to assess this nerve-muscle effect, and a study in dogs did not show this effect. While these animals studies are reassuring, a small risk of developing these changes cannot be ruled out for participants in this study. However, all experience with the clinical use of pyridostigmine in man over the past 20 years has not led to any reports of nerve-muscle difficulties attributed to or resulting from the use of this drug. The 20 years of experience with this drug has been gained by use of pyridostigmine in patients with a nerve-muscle disease known as myasthenia gravis. While there is not extensive information on use of pyridostigmine in healthy persons such as yourself, the long experience with this drug does provide us an indication of the drug's safety and expected side effects.

You are under no obligation to participate in this project. Should you decide not to participate or should you decide to withdraw during the course of the project, your future medical care at Hopkins will not be affected. Benefits to you for participation in this study are primarily financial, but another potential asset is the comprehensive evaluation which accompanies this project, the records of which will be available in the future. You will be paid by check for whatever proportion of the study you complete. Successful completion of the entire study will pay $350. You will be paid by check at the time you leave the hospital.

The records from this research study are kept confidential to the extent allowed by law. Certain regulatory groups are allowed to review them to assure that proper procedures are being followed. In addition to the FDA, authorized representatives from the Army may inspect the records related to your participation in this study.

You are authorized all necessary medical care for injury or disease which is the proximate result of your participation in the research. The medical treatment provided might include, if necessary, laboratory tests, x-rays and other procedures used in diagnosis and treatment. No other compensation for injury is offered.

APPENDIX B

STUDY FLOW SHEET

	Screen	S 1	M 2	TU 3	W 4	Th 5	F 6	Sa 7
Hx + P.E.	x							
Sign Consent	x							
Hematology & Chemistry	x	x	x					x
CPK	x	x	x*	x*		x	x*	x
EKG	x**							
Urinalysis	x							x
Drug Screen		x						
Study Drug Administration***			IV	Os		Om	Om	
Pyridostigmine levels			x	x	x	x	x	x
RBC AChE			x	x	x	x	x	x
Admit to Unit		x						
Discharge from Unit								x

* One level taken before dose administration and one 8 hours after drug administration.

** If not available within past 12 months.

*** IV = intravenous over 8 hours
Os = oral, single dose, fasting
Om = oral, multiple dose

TIMING OF BLOOD SAMPLES FOR IV PYRIDOSTIGMINE

Time After Dosing			Pyridostigmine	Acetylcholinesterase
Hour	Minutes	Sample #'s	Levels	Activity
0			X	X
0	7.5		X	
0	15		X	X
0	22.5		X	
0	30		X	X
1			X	X
1	30		X	X
2			X	X
3			X	X
4			X	X
5			X	X
6			X	X
6	7.5		X	
6	15		X	X
6	22.5		X	
6	30		X	X
7			X	X
7	30		X	X
8			X	X
9			X	X
*10			X	X
12			X	X
14			X	X
24			X	X

* Samples for red blood cell acetylcholinesterase activity and pyridostigmine levels will be obtained after 10 hours only if the previous sample shows inhibition of red blood cell acetylcholinesterase greater than 5%.

TIMING OF BLOOD SAMPLES FOR FASTED ORAL DOSINGS

Time After Dosing			Pyridostigmine	Acetylcholinesterase
Hour	Minutes	Sample #'s	Levels	Activity
0	0	1	X	X
0	15	2	X	X
0	30	3	X	X
0	45	4	X	X
1	0	5	X	X
1	20	6	X	X
1	40	7	X	X
2	0	8	X	X
2	30	9	X	X
3	0	10	X	X
3	30	11	X	X
4	0	12	X	X
5	0	13	X	X
6	0	14	X	X
7	0	15	X	X
8	0	16	X	X
10	0	17	X	X
12	0	18	X	X
14*	0	19	X	X
24*	0	20	X	X

*Samples for red blood cell acetylcholinesterase activity and pyridostigmine levels will be obtained at 14 hours and 24 hours if the previous sample shows inhibition of red blood cell acetylcholinesterase greater than 5%.

TIMING OF BLOOD SAMPLES FOR ORAL PYRIDOSTIGMINE
DURING MULTIPLE DOSING

Time After Dosing			Pyridostigmine	Acetylcholinesterase
Hours	Minutes	Sample #'s	Levels	Activity
0	0	1	X	X
0	15	2	X	X
0	30		X	X
0	45		X	X
1	0		X	X
1	20		X	X
1	40		X	X
2	0		X	X
2	30		X	X
3	0		X	X
3	30		X	X
4	0		X	X
5	0		X	X
6	0		X	X
7	0		X	X
8	0		X	X
*10	0		X	X
*12	0		X	X

* For sustained release-preparation only (given every 12 hours).

APPENDIX C

CLINICAL LABORATORY NORMAL VALUES

The ranges of these values have been determined and are utilized by the Department of Laboratory Medicine of The Johns Hopkins Hospital.

Serum Chemistry	Normal Limits		Units
Tests	Lower	Upper	
Sodium	135	148	mEq/l
Potassium	3.5	5.0	mEq/l
Chloride	96	109	mEq/l
Carbon dioxide	24	30	mEq/l
Serum urea nitrogen	7	22	mg/dl
Creatinine	0.6	1.3	mg/dl
Glucose	70	115	mg/dl
Calcium	8.4	10.5	mg/dl
Total bilirubin	0.2	1.2	mg/dl
Direct bilirubin	0.0	0.4	mg/dl
Total protein	6.0	8.2	g/dl
Albumin	3.5	5.3	g/dl
Aspartate aminotransferase	0	35	IU/l
Alanine aminotransferase	0	30	IU/l
Alkaline phosphatase	0	120	IU/l
Phosphate, inorganic	2.7	4.5	mg/dl
Lactic dehydrogenase	0	220	IU/l
Creatine kinase	0	175	IU/l
Uric Acid	3.5	7.2	mg/dl
Cholesterol	0	199	mg/dl

	Normal Limits		Units
	Lower	Upper	
Hematology Tests			
White blood cells	4,500	11,000	#/mm^3
Red blood cells	4.50	5.90	million/mm^3
Hemoglobin	13.9	16.3	g/dl
Hematocrit	41.0	53.0	%
Platelets	150	350	thousand/mm^3
Reticulocytes	0.5	1.5	%
White Blood Cell Differentials:			
Bands	2	6	%
Segmental Neutrophils	31	76	%
Lymphocytes	24	44	%
Monocytes	2	11	%
Eosinophils	1	4	%
Basophils	0	1	%

APPENDIX D

ASSAY OF ERYTHROCYTE ACETYLCHOLINESTERASE AT THE JOHNS HOPKINS UNIVERSITY DIVISION OF CLINICAL PHARMACOLOGY

The determination of erythrocyte acetylcholinesterase (AChE) follows the protocol established by the US Army Medical Research Institute for Chemical Defense for the determination of erythrocyte AChE.

Principle of Method, Chemicals, Equipment and Materials, Preparation of Reagents

The principles of the methods, the chemicals, materials, and equipment needed, the preparation of reagents and standards are detailed in the Standard Operating Procedure (SOP) of 18 June 1985 of the Analytical Chemistry Branch, USAMR, ICD, Aberdeen Proving Ground, Maryland 21010. These are followed exactly except for preparation of the stock glutathione solution, page 3, number 6 under A. Reagents. The typographical error is corrected and 1.844 g of glutathione, reduced form, (GSH) are dissolved in a total volume of 100ml of EDTA diluent.

Collection and Preparation of Specimen for Sampling

Blood is collected into 3 ml purple topped Vacutainer^{R} (EDTA) through a catheter inserted into an arm vein. After the sample is drawn, it is mixed and brought promptly to the lab at room temperature. One and one half ml of the blood is centrifuged for 2 minutes at 15,000 RPM in an Eppendorf model 5414 centrifuge. After centrifugation all plasma is removed from

the top of the packed erythrocytes with a Pasteur pipet. The erythrocytes are then picked up in a clean Pasteur pipet, starting from the bottom of the centrifuge tube, avoiding drawing air after all the cells have been pipetted. The packed cells are transferred to a 0.5 ml sample cup and are ready for AChE analyses. Sample preparation time is kept to 3 minutes.

Preparation of Standards

Standards are prepared according to the SOP of the ICD.

Preparation of AChE Control Material

A quality control enzyme standard is used to measure assay precision. The enzyme used is electric eel AChE which is diluted in a large volume to a specific activity and frozen in aliquots. An aliquot is used each day the assay is performed. Electric eel samples were assayed in accordance with ICD SOP. See below for specific details.

Analysis Start-Up

ICD SOP is followed. The heating circulator is turned on. We allow temperature to reach 37 degrees C and verify constancy periodically.

Proportioning Pump

ICD SOP is followed. Water with Brij 35 precedes the reagents. A good bubble pattern is verified.

Colorimeter and Recorder

ICD SOP is followed. Lamp warmup is at least 10 minutes. Once reagents are running, the recorder is zeroed for no signal and full scale. Speed is set to 1.0 cm/min.

Sampler

ICD SOP is followed. Proper operation of sampler is verified prior to running any samples.

Analyses

Recorder baseline is set to zero.

Manifold has been modified so that the substrate blank side pumps saline continuously. Erythrocytes enter only one side and one photo cell. At the end of the day, a substrate blank is run by placing the substrate line into saline and measuring the activity of the erythrocytes without a substrate. ICD SOP is followed.

Incubation time is measured. The length of time it takes erythrocytes to travel from the point where substrate is added to the sample stream until exit from the dialyzer is defined as the incubation time.

GSH standards and electric eel AChE are assayed first. A 60 micromoles/ml (u moles/ml) GSH standard is assayed followed by 15, 30, and 45 u moles/ml GSH standards, and a 1:1 dilution of the electric eel quality control. Machine sensitivity is adjusted using the STD CAL knob so that the 60 u moles/ml standard reads 80 to 90 on the recorder scale. Two standard curves and a minimum 3 electric eel samples are assayed prior to assay of erythrocyte samples. Erythrocyte samples are separated by at least two saline cups. Machine performance is standardized periodically throughout the day by running standard curves and electric eel samples. Gain is decreased if the 60 u moles/ml GSH rises above 90. Where possible, standards are assayed hourly and

erythrocyte samples measured in duplicate.

Shut Down SOP

ICD is followed. Care is taken to dry the tubes and to insure that they are in a relaxed position.

Data Reduction

AChE activity is determined as follows:

The concentration of each GSH standard is divided by the peak height of the deflection produced. This value is then divided by the time of incubation in minutes to produce a factor of u moles/ml/min/chart unit. The individual factors from each standard in the curve are averaged. The average factor, u moles/ml/min/chart unit, is used to convert the deflection produced by samples to AChE activity, u moles/ml/min.

ASSAY PERFORMANCE AT JOHNS HOPKINS HOSPITAL

Quality Control

A standard solution of electric eel acetylcholinesterase is measured several times on the days that assays are performed.

The current batch of JHH electric eel was prepared on 14 October 1985 using Sigma Co, Type VI-S Cholinesterase, Acetyl (catalog #C3389) from electric eel, Lot #83F8100. One 10,000 unit vial was diluted in Tris buffer containing 1% bovine serum albumin. The dilution target was such that a one to one dilution of the solution should contain 15.0 u moles/ml/min of cholinesterase activity.

After the dilution, aliquotting and freezing of the cholinesterase solution was performed, it was discovered that the

colorimeter was not functioning correctly and that the GSH standards were in error. New standards were made; the colorimeter was repaired. The instrument was calibrated with GSH standards and the Aberdeen quality control standard. The latter's activity was 7.46 u moles/ml/min, within the range at ICD, Aberdeen.

The assay was used on 13 different days. On each day an aliquot of the electric eel quality control standard was thawed and assayed several times. The overall statistics of 127 samples from the 13 aliquots show mean activity 9.88 u moles/ml/min with a standard deviation of 0.27 u moles/ml/min. The coefficient of variation is 2.7%. Looking only at the means of the assays in a single day, the activity of the quality control is 9.91 ± 0.23 u moles/ml/min (C.V. = 2.3%).

Precision

Blood samples were drawn from 6 study subjects prior to drug administration. Each sample was assayed on several occasions. The results are tabulated in the Table.

The intra-assay coefficient of variation ranged from 0.5 to 3.5%, with a mean of 2.5%. Day to day variations in the measurement of a subject's drug free erythrocyte AChE ranged from 0.14 to 0.67 u moles/ml/min or 1.08 to 5.48% of the value on a single day. This variation is about twice the intra-assay variation and suggests that either the inter-assay variation is greater than that of the intra-assay and/or the activity of erythrocyte AChE drawn from a single subject on different days varies to a small degree.

With a coefficient of variation of 2.5% in the assay, inhibition of enzyme activity of 5% or more probably represents real inhibition by pyridostigmine, not random variation due to the assay.

TABLE - APPENDIX D

ERYTHROCYTE ACETYLCHOLINESTERASE ACTIVITY IN 6 NORMAL VOLUNTEERS

Intra-Assay and Inter-Assay Results

Subject	Day	n	AChE u moles/ml/min (± S.D.)	C.V. %	b-a u moles/ml/min	(b-a)/b %
A	a	7	12.90 ± 0.31	2.4	0.14	1.08
	b	9	13.04 ± 0.31	2.4		
B	a	10	13.82 ± 0.37	2.7	0.66	4.78
	b	8	14.48 ± 0.55	3.8		
C	a	8	12.59 ± 0.36	2.9	0.69	5.48
	b	8	13.28 ± 0.31	2.3		
D	a	4	13.20 ± 0.52	3.9	0.67	5.08
	b	5	13.87 ± 0.07	0.5		
E	a	4	13.35 ± 0.43	3.2	0.51	3.82
	b	6	13.86 ± 0.33	2.4		
F	a	4	14.26 ± 0.36	2.5	0.60	4.21
	b	6	14.86 ± 0.20	1.3		

APPENDIX E

```
MODEL
TEMP
T=X
V=P(1)
K10=P(2)
K12=P(3)
K21=P(4)
D=CON(1)
TI=CON(2)
DEL=T-TI
TSTAR=MAX(0,DEL)
R1=DSQRT((K12+K21+K10)**2 - (4*K21*K10))
ALPHA=((K12+K21+K10) + R1)/2
BETA=((K12+K21+K10) - R1)/2
A=(D/TI/V)*(K21-ALPHA)/(ALPHA-BETA)/ALPHA
B=-1*(D/TI/V)*(K21-BETA)/(ALPHA-BETA)/BETA
END
FUNC1
F=A*(DEXP(-ALPHA*T) - DEXP(-ALPHA*TSTAR)) +  &
     B*(DEXP(-BETA*T) - DEXP(-BETA*TSTAR))
END
SECO
S(1)=D/V/K10
S(2)=-DLOG(.5)/K10
S(3)=ALPHA
S(4)=BETA
S(5)=-DLOG(.5)/ALPHA
S(6)=-DLOG(.5)/BETA
S(7)=A*(DEXP(-ALPHA*TI) - 1.0) +     &
     B*(DEXP(-BETA*TI) - 1.0)
REMA S8 AND S9 ARE THE INTERCEPTS FOLLOWING IV INJECTION
S(8)= -1.0 * A * ALPHA * TI
S(9)= -1.0 * B * BETA * TI
END
EOM
NPARM 4
NSEC 9
PNAMES 'VOLUME', 'K10', 'K12', 'K21'
SNAMES 'AUC', 'K10-HL', 'ALPHA', 'BETA', 'ALPHA-HL', 'BETA-HL', &
       'CMAX', 'A', 'B'
NCON 2
CONS 4272,6
INIT 11.33,3.39,1.60,1.02
WEIGHT -1
NOBS 18
OUTPUT 'A:O14P02.A2A'
DATA 'A:14P02A.D'
BEGIN
FINISH
^Z
```

```
MODEL
TEMP
T=X
V=CON(1)
K10=CON(2)
K12=CON(3)
K21=CON(4)
K01STDFA=P(1)
TLAGFA=P(2)
FSTDFAST=P(3)
TFA=MAX(0,T-TLAGFA)
DSTD=CON(5)
R1=DSQRT((K12+K21+K10)**2 - (4*K21*K10))
ALPHA=((K12+K21+K10) + R1)/2
BETA=((K12+K21+K10) - R1)/2
AFA=(FSTDFAST*DSTD/V)*K01STDFA*(K21-ALPHA)/(ALPHA-BETA)/(ALPHA-K01STDFA)
BFA=-1*(FSTDFAST*DSTD/V)*K01STDFA*(K21-BETA)/(ALPHA-BETA)/(BETA-K01STDFA)
CFA=(FSTDFAST*DSTD/V)*K01STDFA*(K21-K01STDFA)/(BETA-K01STDFA)/ &
  (ALPHA-K01STDFA)
END
FUNC1
F=MAX(0,AFA*DEXP(-ALPHA*TFA) + BFA*DEXP(-BETA*TFA) +  &
CFA*DEXP(-K01STDFA*TFA))
END
SECO
S(1)=ALPHA
S(2)=BETA
S(3)=-DLOG(.5)/ALPHA
S(4)=-DLOG(.5)/BETA
S(5)= FSTDFAST*DSTD/V/K10
S(6)=-DLOG(.5)/K01STDFA
END
EOM
NPARM 3
NSEC 6
PNAMES  'K01STDFA', 'TLAGFA',,'FSTDFAST',
SNAMES  'ALPHA', 'BETA', 'ALPHA-HL', 'BETA-HL', 'AUCFAST',  &
'ABS-HLFA'
NCON 5
CONS 11.65,3.39,1.60,1.02,20889
INIT .25,.01,.16
LOWER 0,0,0,
UPPER 100,100,1
METHOD 1
WEIGHT 0
NFUNC 1
NOBS 14
OUTPUT 'A:O14P02.B'
DATA 'A:14P02B.D'
BEGIN
NEWP 1,1
TITLE
WEIGHT -1
WEIGHT -1
BEGIN
FINISH^Z
```

```
MODEL
TEMP
T=X
V=CON(1)
K10=CON(2)
K12=CON(3)
K21=CON(4)
K01STDFE=P(1)
TLAGFE=P(2)
FSTDFED=P(3)
TFE=MAX(0,T-TLAGFE)
DSTD=CON(5)
R1=DSQRT((K12+K21+K10)**2 - (4*K21*K10))
ALPHA=((K12+K21+K10) + R1)/2
BETA=((K12+K21+K10) - R1)/2
AFE=(FSTDFED*DSTD/V)*K01STDFE*(K21-ALPHA)/(ALPHA-BETA)/(ALPHA-K01STDFE)
BFE=-1*(FSTDFED*DSTD/V)*K01STDFE*(K21-BETA)/(ALPHA-BETA)/(BETA-K01STDFE)
CFE=(FSTDFED*DSTD/V)*K01STDFE*(K21-K01STDFE)/(BETA-K01STDFE)/ &
  (ALPHA-K01STDFE)
END
FUNC1
F=MAX(0,AFE*DEXP(-ALPHA*TFE) + BFE*DEXP(-BETA*TFE) +  &
CFE*DEXP(-K01STDFE*TFE))
END
SECO
S(1)=ALPHA
S(2)=BETA
S(3)=-DLOG(.5)/ALPHA
S(4)=-DLOG(.5)/BETA
S(5)= FSTDFED*DSTD/V/K10
S(6)=-DLOG(.5)/K01STDFE
END
EOM
NPARM 3
NSEC 6
PNAMES  'K01STDFE', 'TLAGFE',,'FSTDFED',
SNAMES  'ALPHA', 'BETA', 'ALPHA-HL', 'BETA-HL', 'AUCFED',  &
'ABS-HLFE'
NCON 5
CONS 11.65,3.39,1.60,1.02,20889
INIT .27,.35,.14
LOWER 0,0,0,
UPPER 100,100,1
METHOD 1
WEIGHT 0
NFUNC 1
NOBS 15
OUTPUT 'A:014P02.C'
DATA 'A:14P02C.D'
BEGIN
NEWP 1,1
TITLE
WEIGHT -1
WEIGHT -1
BEGIN
FINISH
```

```
MODEL
TEMP
T=X
V=P(1)
K10=P(2)
K12=P(3)
K21=P(4)
K01STDFA=P(5)
K01STDFE=P(6)
TLAGFA=P(7)
TLAGFE=P(8)
FSTDFAST=P(9)
FSTDFED=P(10)
TFA=MAX(0,T-TLAGFA)
TFE=MAX(0,T-TLAGFE)
DIV=CON(1)
TI=CON(2)
DSTD=CON(3)
DEL=T-TI
TSTAR=MAX(0,DEL)
R1=DSQRT((K12+K21+K10)**2 - (4*K21*K10))
ALPHA=((K12+K21+K10) + R1)/2
BETA=((K12+K21+K10) - R1)/2
A=(DIV/TI/V)*(K21-ALPHA)/(ALPHA-BETA)/ALPHA
B=-1*(DIV/TI/V)*(K21-BETA)/(ALPHA-BETA)/BETA
AFA=(FSTDFAST*DSTD/V)*K01STDFA*(K21-ALPHA)/(ALPHA-BETA)/(ALPHA-K01STDFA)
BFA=-1*(FSTDFAST*DSTD/V)*K01STDFA*(K21-BETA)/(ALPHA-BETA)/(BETA-K01STDFA)
CFA=(FSTDFAST*DSTD/V)*K01STDFA*(K21-K01STDFA)/(BETA-K01STDFA)/ &
  (ALPHA-K01STDFA)
AFE=(FSTDFED*DSTD/V)*K01STDFE*(K21-ALPHA)/(ALPHA-BETA)/(ALPHA-K01STDFE)
BFE=-1*(FSTDFED*DSTD/V)*K01STDFE*(K21-BETA)/(ALPHA-BETA)/(BETA-K01STDFE)
CFE=(FSTDFED*DSTD/V)*K01STDFE*(K21-K01STDFE)/(BETA-K01STDFE)/ &
  (ALPHA-K01STDFE)
END
FUNC1
F=A*(DEXP(-ALPHA*T) - DEXP(-ALPHA*TSTAR)) +  &
     B*(DEXP(-BETA*T) - DEXP(-BETA*TSTAR))
END
FUNC2
F=MAX(0,AFA*DEXP(-ALPHA*TFA) + BFA*DEXP(-BETA*TFA) +  &
CFA*DEXP(-K01STDFA*TFA))
END
FUNC3
F=MAX(0,AFE*DEXP(-ALPHA*TFE) + BFE*DEXP(-BETA*TFE) +  &
CFE*DEXP(-K01STDFE*TFE))
END
SECO
S(1)=DIV/V/K10
S(2)=-DLOG(.5)/K10
S(3)=ALPHA
S(4)=BETA
S(5)=-DLOG(.5)/ALPHA
S(6)=-DLOG(.5)/BETA
S(7)=A*(DEXP(-ALPHA*TI) - 1.0) +      &
     B*(DEXP(-BETA*TI) - 1.0)
REMA S8 AND S9 ARE THE INTERCEPTS FOLLOWING IV INJECTION
S(8)= -1.0 * A * ALPHA * TI
S(9)= -1.0 * B * BETA * TI
S(10)= FSTDFAST*DSTD/V/K10
S(11)= FSTDFED*DSTD/V/K10
S(12)=-DLOG(.5)/K01STDFA
S(13)=-DLOG(.5)/K01STDFE
END
EOM
NPARM 10
NSEC 13
PNAMES 'VOLUME', 'K10', 'K12', 'K21', 'K01STDFA','K01STDFE', &
    'TLAGFA','TLAGFE','FSTDFAST','FSTDFED'
SNAMES 'AUC', 'K10-HL', 'ALPHA', 'BETA', 'ALPHA-HL', 'BETA-HL', &
       'CMAX', 'A', 'B','AUCFAST','AUCFED','ABS-HLFA','ABS-HLFE'
NCON 3
CONS 4272,6,20889
INIT 11.18,3.46,1.67,1.33,0.26,0.27,0.0001,0.35,0.157,0.139
LOWER 0,0,0,0,0,0,0,0,0,0,
UPPER 1000,100,100,100,100,100,100,100,1,1,
METHOD 1
WEIGHT 0
NFUNC 3
NOBS 18,14,15
OUTPUT 'A:O14P02.ABC'
DATA 'A:14P02ABC.D'
BEGIN
FINISH
^Z
```

APPENDIX F

THE JOHNS HOPKINS UNIVERSITY

SCHOOL OF MEDICINE

DIVISION OF
CLINICAL PHARMACOLOGY
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Task Order #14

Subject #	Weight (kg)	Adverse Events	Relation
1	76.5	---	
2	71.0	Ringing in ears	Possible
3	76.0	Slight rash on forehead	Remote
4	63.1	---	
5	66.8	Cramps, flatus, heartburn	Possible
6	77.0	Broken tooth	Remote
7	76.0	Headache, nausea, diarrhea	Possible
8	82.5	Hunger pangs, "rumbling in stomach"	Possible
9	78.0	Flatus, headache	Probable
10	71.0	---	
11	66.4	---	
12	83.2	---	
13	83.4	---	
14	84.4	---	
15	100.3	Gastric queasiness	Possible
16	63.9	---	

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APPENDIX G

Adverse Events

Definitions of Relationship to Test Drug

Probably Related (must have first three)

This category applies to those adverse experiences which are considered, with a moderate degree of certainty, to be related to the test drug. An adverse experience may be considered probably related to the drug if:

1. It follows a reasonable temporal sequence from administration of the drug.

2. It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.

3. It disappears or decreases on cessation or reduction in dose. There are important exceptions when an adverse experience does not disappear upon discontinuation of the drug, yet drug relatedness clearly exists; e.g., 1) bone marrow depression; 2) fixed drug eruptions; and 3) tardive dyskinesias.

4. It follows a known pattern of response to the suspected drug.

Possibly Related (must have first two)

This category applies to those adverse experiences in which the connection with the test drug administration appears unlikely but cannot be ruled out with certainty. An adverse experience may be considered possibly related to the drug if:

1. It follows a reasonable temporal sequence from administration of the drug.

2. It could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the volunteer.

3. It follows a known pattern to the suspected drug.

Adverse Events

Remote

This category is applicable to those adverse experiences which are judged to be clearly and incontrovertibly due to extraneous causes (disease, environment, etc.) and do not meet the criteria for drug relationship listed under Definitely, Probably or Possibly Related. In such cases there is no conceivable way in which the test drug could be implicated; the event is associated with a known underlying condition or is physiologically impossible as a drug side effect; or is so remote from drug exposure as to be impossible.

APPENDIX H

Personnel Receiving Contract Support

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APPENDIX I

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